Safety Assessment of Transgenic Organisms

OECD CONSENSUS DOCUMENTS

Volume 3



OECD

Safety Assessment of Transgenic Organisms

OECD CONSENSUS DOCUMENTS

Volume 3



This work is published on the responsibility of the Secretary-General of the OECD. The opinions expressed and arguments employed herein do not necessarily reflect the official views of the Organisation or of the governments of its member countries.

Please cite this publication as:
OECD (2010), *Safety Assessment of Transgenic Organisms: OECD Consensus Documents: Volume 3*, OECD Publishing.
http://dx.doi.org/10.1787/9789264095434-en

ISBN 978-92-64-09542-7 (print)
ISBN 978-92-64-09543-4 (PDF)

Corrigenda to OECD publications may be found on line at: *www.oecd.org/publishing/corrigenda*.

© OECD 2010

You can copy, download or print OECD content for your own use, and you can include excerpts from OECD publications, databases and multimedia products in your own documents, presentations, blogs, websites and teaching materials, provided that suitable acknowledgment of OECD as source and copyright owner is given. All requests for public or commercial use and translation rights should be submitted to *rights@oecd.org*. Requests for permission to photocopy portions of this material for public or commercial use shall be addressed directly to the Copyright Clearance Center (CCC) at *info@copyright.com* or the Centre français d'exploitation du droit de copie (CFC) at *contact@cfcopies.com*.

Foreword

Genetically engineered crops (also known as transgenic crops) have been approved for commercial release in an increasing number of countries, for planting or for use as commodities. Genetically engineered varieties of over a dozen different plant species have received regulatory approval in several OECD and non-OECD countries from all regions of the world, the large majority of plantings being for soybean, maize, cotton and rapeseed (canola), as outlined in *The Bioeconomy to 2030: Designing a Policy Agenda* (OECD, 2009). During the period from 1996 to 2009, for example, there was an almost eighty-fold increase in the area grown with transgenic crops worldwide, reaching 134 million hectares in 2009, as mentioned in *Global Status of Commercialized Biotech/GM Crops* (James, 2009). Such approvals usually follow a science-based risk/safety assessment.

The environmental safety/risks of transgenic organisms are normally based on the information on the characteristics of the host organism, the introduced traits, the environment into which the organism is introduced, the interaction between these, and the intended application. The OECD's Working Group on Harmonisation of Regulatory Oversight in Biotechnology decided at its first session, in June 1995, to focus its work on identifying parts of this information, which could be commonly used in countries for environmental safety/risk assessment to encourage information sharing and prevent duplication of effort among countries. Biosafety Consensus Documents are one of the major outputs of its work.

Biosafety Consensus Documents are intended to be a "snapshot" of current information on a specific host organism or trait, for use during regulatory assessments. They are not intended to be a comprehensive source of information on everything that is known about a specific host or trait, but they do address the key or core set of issues that member countries believe are relevant to risk/safety assessment. Several non-member economies, as well as other international organisations, are associated with the work and share their expertise. The information collated in the Consensus Documents is said to be mutually acceptable among member countries and also other countries wishing to use them for their assessment process.

To date, 38 Biosafety Consensus Documents have been published. They include documents which address the biology of crops, trees and micro-organisms as well as those which address specific traits which are used in transgenic crops. In addition, documents of broader nature aiming to facilitate harmonisation have been developed: *Designation of a Unique Identifier for Transgenic Plants* (2002, revised in 2006); and *Molecular Characterisation of Plants Derived from Modern Biotechnology* (2010).

Volumes 3 and 4 of this publication contain a compilation of those Biosafety Consensus Documents published between September 2006 and September 2010. These volumes also include two previously published presentation texts (slightly updated since Volumes 1 and 2):

- *An Introduction to the Biosafety Consensus Documents of OECD's Working Group for Harmonisation in Biotechnology* explains the purpose of the documents and how they are relevant to risk/safety assessment. It also describes the process by which the documents are drafted, using a "lead country" approach.

- Then, the *Points to Consider for Consensus Documents on the Biology of Cultivated Plants* offer a structured checklist of points for authors to consider when drafting, or to experts evaluating a Consensus Document. Each point is described for its relevance to risk/safety assessment.

Along with Volumes 1 and 2, the present publication offers ready access to those Consensus Documents which have been published thus far. As such, it should be of value to applicants for commercial uses of transgenic crops, regulators in national authorities as well as the wider scientific community.

As each of the Consensus Documents may be updated in the future as new knowledge becomes available, users of this book are encouraged to provide any information or opinions regarding the contents of the Consensus Documents or indeed, OECD's other harmonisation activities. Comments can be provided at: *biosafety@oecd.org*.

The published Consensus Documents are also available individually from the OECD's Biotrack website, at no cost (www.oecd.org/biotrack).

Acknowledgements

This book is the result of the common effort of the participants in the OECD's Working Group on Harmonisation of Regulatory Oversight in Biotechnology. Each section is composed of a "Consensus Document" which was prepared under the leadership of a participating country or countries, as listed at the end of this volume. During their successive draftings, valuable inputs and suggestions for the documents were provided by a number of delegates and experts in the Working Group, being from OECD Members, non member economies and observer organisations.

Each Consensus Document was issued individually, as soon as finalised and agreed for declassification, by the OECD Environment, Health and Safety Division in the *Series on Harmonisation of Regulatory Oversight in Biotechnology.* Volumes 3 and 4 of this publication, containing the 2006-2010 Consensus Documents, were prepared and edited by Bertrand Dagallier and Carina Arambula, under the supervision of Peter Kearns, at the EHS Division, OECD Environment Directorate.

Table of contents

Foreword **3**

Table of contents **5**

Introduction to the biosafety consensus documents **7**

Présentation des documents de consensus sur la sécurité biologique **17**

Points to consider for consensus documents on the biology of cultivated plants **27**

Part 1. **Consensus documents on the biology of trees** **39**

Section 1. Western white pine (*Pinus monticola*) 40

Section 2. Jack pine (*Pinus banksiana*) 57

Section 3. Native north american larches: subalpine larch (*Larix lyallii*), western larch (*L. occidentalis*), and tamarack (*L. laricina*) 91

Section 4. Douglas-Fir (*Pseudotsuga menziesii*) 120

Section 5. Lodgepole pine (*Pinus contorta*) 152

Section 6. Black spruce (*Picea mariana*) 176

Part 2. **Consensus document on safety information on traits** **217**

Section 1. Safety information on transgenic plants expressing *Bacillus thuringiensis* - Derived insect control protein 218

Part 3. **Documents to facilitate harmonised safety assessments** **299**

Section 1. OECD guidance for the designation of a unique identifier for transgenic plants ... 300

Section 2 Molecular characterisation of plants derived from modern biotechnology 305

List of OECD consensus documents 1996-2010 ..... **319**

Introduction to the biosafety consensus documents

1. About OECD's Working Group for biosafety

The OECD's *Working Group on Harmonisation of Regulatory Oversight in Biotechnology* (the Working Group) comprises delegates from the 33 member countries of OECD and the European Commission. Typically, delegates are from those government ministries and agencies, which have responsibility for the environmental risk/safety assessment of products of modern biotechnology. The Working Group also includes a number of observer delegations and invited experts who participate in its work, such as Argentina; the Russian Federation; the United Nations Environment Programme (UNEP) and; the Secretariat of the Convention on Biological Diversity (SCBD); the Food and Agriculture Organization of the United Nations (FAO), the United Nations Industrial Development Organisation (UNIDO); and the Business and Industry Advisory Committee to the OECD (BIAC). In recent years, with the increasing use of biotech products in many regions of the world together with the development of activities relating to tropical and subtropical species, there has been increased participation of non-member economies including Brazil, Cameroon, China, Estonia, India, the Philippines and South Africa.

2. Regulatory harmonisation

The Working Group was established in 1995[1] at a time when the first commercial transgenic crops were being considered for regulatory approval in a number of OECD member countries. From the beginning, one of the group's primary goals was to promote international regulatory harmonisation in biotechnology among members. Regulatory harmonisation is the attempt to ensure that the information used in risk/safety assessments, as well as the methods used to collect such information, are as similar as possible. It could lead to countries recognising or even accepting information from one anothers' assessments. The benefits of harmonisation are clear. It increases mutual understanding among countries, which avoids duplication, saves on scarce resources and increases the efficiency of the risk/safety assessment process. This in turn improves safety, while reducing unnecessary barriers to trade (OECD, 2000).

3. The need for harmonisation activities at OECD

The establishment of the Working Group and its programme of work followed a detailed analysis by member countries of whether there was a need to continue work on harmonisation in biotechnology at OECD, and if so, what it should entail. This analysis was undertaken by the *Ad Hoc* Group for Environmental Aspects of Biotechnology (established by the Joint Meeting[2]), in 1994 mainly.

The *Ad Hoc* Group took into consideration, and built upon, the earlier work at OECD which began in the mid-1980s. Initially, these OECD activities focused on the environmental and agricultural implications of field trials of transgenic organisms, but this was soon followed by a consideration of their large-scale use and commercialisation. (A summary of this extensive body of work is found in Appendix I.)

4. Key background concepts and principles

The *Ad Hoc* Group took into account previous work on risk analysis that is summarised in *Safety Considerations for Biotechnology: Scale-up of Crop Plants* (OECD, 1993a). The following quote gives the flavour: "Risk/safety analysis is based on the characteristics of the organism, the introduced trait,

1 . The original title of the Working Group was the "Expert Group for the Harmonisation of Regulatory Oversight in Biotechnology". It became an OECD Working Group in 1998.

2 . The Joint Meeting was the supervisory body of the *Ad Hoc* Group and, as a result of its findings, established the Working Group as a subsidiary body. Today, its full title is the Joint Meeting of the Chemicals Committee and the Working Party on Chemical, Pesticides and Biotechnology.

the environment into which the organism is introduced, the interaction between these, and the intended application." This body of work has formed the basis for environmental risk/safety assessment that is now globally accepted. In considering the possibilities for harmonisation, the *Ad Hoc* Group paid attention to these characteristics and the information used by risk/safety assessors to address them.

This was reinforced by the concept of familiarity, also elaborated in the above-mentioned document (OECD, 1993a). This concept "is based on the fact that most genetically engineered organisms are developed from organisms such as crop plants whose biology is well understood". "Familiarity allows the risk assessor to draw on previous knowledge and experience with the introduction of plants and micro-organisms into the environment." For plants, familiarity takes account of a wide-range of attributes including, for example, knowledge and experience with "the crop plant, including its flowering/reproductive characteristics, ecological requirements, and past breeding experiences" (OECD ,1993a – see also Appendix I for a more detailed description). This illustrates the role of information related to the biology of the host organism as a part of an environmental risk/safety assessment.

The *Ad Hoc* Group also considered the document *Traditional Crop Breeding Practices: An Historical Review to Serve as a Baseline for Assessing the Role of Modern Biotechnology* (OECD, 1993b) which focuses on host organisms. It presents information on 17 different crop plants, which are used (or are likely to be used) in modern biotechnology. It includes sections on phytosanitary considerations in the movement of germplasm and on current uses of these crop plants. There is also a detailed section on current breeding practices.

5. A common approach to risk/safety assessment

An important aspect for the *Ad Hoc* Group was to identify the extent to which member countries address the same questions and issues during risk/safety assessment. Big differences would mean difficulties in working towards harmonisation, while a high level of similarity would suggest it more feasible.

This point was resolved by two studies considered by the *Ad Hoc* Group: one covered crop plants (OECD, 1995a; 1995b) while the other concerned micro-organisms (OECD, 1995c; 1996). Both studies involved a survey with national authorities responsible for risk/safety assessment. The aim was to identify the questions they address during the assessment process (as outlined in national laws/regulations/guidancetexts) in order to establish the extent of similarity among national authorities. The studies used the information provided in the OECD's *Blue Book* on Recombinant DNA Safety Considerations (OECD, 1986) as a reference point, in particular, the sections covering: i) General Scientific Considerations; ii) Human Health Considerations; and iii) Environmental and Agricultural Considerations (appendices b, c and d). Both studies showed a remarkably high degree of similarity among countries in the questions/issues addressed in risk/safety assessment.

6. The emergence of the concept of consensus documents

The Working Group was therefore established in the knowledge that national authorities have much in common, in terms of the questions/issues addressed, when undertaking risk/safety assessment. It also took into account those characteristics identified as part of the assessment (*i.e. the organism*, *the introduced trait* and *the environment*) around which harmonisation activities could focus.

It was further recognised that much of the information used in risk/safety assessment relating to the biology of host organisms (crop plants, trees, animals or micro-organisms) would be similar or virtually the same in all assessments involving the same organism. In other words, the questions addressed during risk/safety assessment which relate to the biology of the organism --for example, the potential for gene transfer within the crop plant species, and among related species, as well as the potential for weediness--

remain the same for each application involving the same host species. This also applies to some extent to information related to introduced traits.

Consequently, the Working Group evolved the idea of compiling information common to the risk/safety assessment of a number of transgenic products, and decided to focus on two specific categories: the biology of the host species; and traits used in genetic modifications. The aim was to encourage information sharing and prevent duplication of effort among countries by avoiding the need to address the same common issues in applications involving the same organism or trait. It was recognized that biology and trait consensus documents could be agreed upon relatively quickly by the member countries (within a few years). This compilation process was quickly formalised in the drafting of Consensus Documents.

7. The purpose of consensus documents

The Consensus Documents are not intended to be a substitute for a risk/safety assessment, because they address only a part of the necessary information. Nevertheless, they should make an important contribution to environmental risk/safety assessment.

Consensus Documents are intended to be a "snapshot" of current information, for use during the regulatory assessment of products of biotechnology. They are not intended to be a comprehensive source of information covering the full knowledge about a specific host organism or trait; but they address – on a consensus basis – the key or core set of issues that countries believe to be relevant to risk/safety assessment.

The aim of the documents is to share information on these key components of an environmental safety review in order to prevent duplication of effort among countries. The documents are envisaged to be used: a) by applicants as information to be given in applications to regulatory authorities; b) by regulators as a general guide and reference source in their reviews; and c) by governments for information sharing, research reference and public information.

Originally, it was said that the information in the Consensus Documents is intended to be *mutually recognised* or *mutually acceptable* among OECD Members, though the precise meaning of these terms is still open for discussion. During the period of the *Ad Hoc* Group and the early days of the Working Group (1993-1995), the phrase *Mutual Acceptance of Data* was discussed. This concept, borrowed from OECD's Chemicals Programme, involves OECD Council Decisions that have legally binding implications for member countries. In the case of the Consensus Documents there has never been legally binding commitment to use the information they contain, though the Working Group is interested in enhancing the commitment of countries to make use of the documents. Participation in the development of documents, and the intention by countries to use the information, is done in "good faith." It is expected, therefore, that reference will be made to relevant Consensus Documents during risk/safety assessments. As these documents are publicly-available tools, they can be of interest for any country wishing to use them in national assesments.

8. The process through which consensus documents are initiated and brought to publication

There are a number of steps in the drafting of a specific Consensus Document. The first step occurs when a delegation, in a formal meeting of the Working Group, makes a proposal to draft a document on a new topic, typically a crop species or a trait. If the Working Group agrees to the proposal, a provisional draft is prepared by either a single country or two or more countries working together. ("lead country approach"). Typically, the lead country(ies) has had experience with the concerned crop or trait and is able to draw on experts to prepare a provisional draft.

The provisional draft is first reviewed by the Bureau of the Working Group [3] to ensure that the document addresses the range of issues normally covered by Consensus Documents and is of sufficiently high quality to merit consideration by the Working Group as a whole.

Based on the comments of the Bureau, a first draft is prepared for consideration by the full Working Group. This is the opportunity for each delegation to review the text and provide comments based on their national experiences. Inputs are incorporated in a second draft, which is again circulated to the Working Group. At this point, the Working Group may be asked to recommend that the document be declassified. Such a recommendation is only forthcoming when all delegations have come to a consensus that the document is complete and ready for publication. Sometimes, however, the text may need a third or even more discussions in the Working Group before a declassification could be contemplated.

When the Working Group has agreed to recommend a document for declassification, it is forwarded to the supervisory Committee, the Joint Meeting, which is invited to declassify the document. Following the agreement of the Joint Meeting, the document is then published.

It is important to note that the review of Consensus Documents is not limited to formal meetings of the Working Group. Much discussion also occurs through electronic means, especially *via* the protected website dedicated to the Working Group. This enables a range of experts to have input into drafts.

For a number of documents, it has also been necessary to include information from non-member countries. This wider share of expertise has become increasingly important in recent years with the development of of activities relating to tropical and subtropical species. And this has been particularly true in the case of crop plants where the centre of origin and diversity occurs in a non-member country(ies). In these cases, UNEP, UNIDO and FAO have assisted in the preparation of documents by identifying experts from concerned countries. For example, this occurred with the Consensus Document on the Biology of Rice.

9. Current and future trends in the Working Group

The Working Group continues its work on the preparation of specific Consensus Documents, and on the efficiency of the process by which they are developed. An increasingly large number of crops and other host species (trees, animals, micro-organisms) are being modified, for an increasing number of traits, and the Working Group aims to fulfil the current needs and be prepared for emerging topics.

At the OECD Workshop on Consensus Documents and Future Work in Harmonisation, held in Washington DC in October 2003, the Working Group considered how to set priorities for drafting future Consensus Documents among the large number of possibilities. The Workshop also recognised that published Consensus Documents may be in need of review and updating from time to time, to ensure that they include the most recent information. The Working Group is considering these aspects on a regular basis when planning future work. For the preparation of future documents, the Workshop identified the usefulness of developing a standardised structure of Consensus Documents. The Working Group contemplated to develop, firstly, a "*Points to Consider*" document for the biology documents and then that of the trait documents. The text on biology documents, published in 2006, is reproduced in the following section of this publication.

3 . The Bureau comprises the Chair and vice-Chairs of the Working Group. The Bureau is elected by the Working Group once per year. At the time of preparing this publication - Vol. 3 and 4, the Chair is from the United States, and the vice-Chairs from Canada, Finland, Japan and the Netherlands.

10. The OECD Task Force for the Safety of Novel Foods and Feeds

The *OECD Task Force for the Safety of Novel Foods and Feeds* (Task Force), established in 1999, addresses aspects of the assessment of human food and animal feed derived from genetically engineered crops. As with the Working Group, the main focus of the Task Force work is to ensure that the types of information used in risk/safety assessement, as well as the methods to collect such information, are as similar as possible amongst countries. The approach is to compare transgenic crops and derived products with similar conventional ones that are already known and considered safe because of recognised experience in their use. Harmonised methods and the sharing of information are facilitated through the Task Force activities.

Similarly to the biosafety programme, the main outcome of the foods and feeds programme is the set of Consensus Documents on compositional considerations of new varieties of specific crops. The Task Force documents compile a common base of scientific information on the major components of crop plants, such as key nutrients, toxicants, anti-nutrients and allergens. These documents constitute practical tools for regulators and risk/safety assessors dealing with these new varieties, with respect to foods and feeds. To date, 20 Consensus Documents have been published on major crops and on general considerations for facilitating harmonisation. They constitute the Series on the Safety of Novel Foods and Feeds which is also available on the OECD's website at no cost (www.oecd.org/biotrack).

The publication of the full Foods and Feeds Series in a single document is contemplated for 2011.

The Working Group and the Task Force are implementing closely-related and complementary programmes, focused on environmental aspects for the first one, on food and feed aspects for the second. Their cooperation on issues of common interest resulted recently in the first Consensus Document developed jointly by the two bodies, the *Consensus Document on Molecular Characterisation of Plants Derived from Modern Biotechnology*, published in September 2010. The document is included in Volume 3 of this publication.

Appendix I

OECD biosafety principles and concepts developed prior to the Working Group (1986-1994)

Since the mid-1980s the OECD has been developing harmonised approaches to the risk/safety assessment of products of modern biotechnology. Prior to the establishment of the Working Group, OECD published a number of reports on safety considerations, concepts and principles for risk/safety assessment as well as information on field releases of transgenic crops, and a consideration of traditional crop breeding practices. This Appendix notes some of the highlights of these achievements that were background considerations in the establishment of the Working Group and its development of Consensus Documents.

Underlying scientific principles

In 1986, OECD published its first safety considerations for genetically engineered organisms (OECD, 1986). These included the issues relevant to human health, the environment and agriculture that might be considered in a risk/safety assessment. In its recommendations for agricultural and environmental applications, it suggested that risk/safety assessors:

- "Use the considerable data on the environmental and human health effects of living organisms to guide risk assessments;
- Ensure that recombinant DNA organisms are evaluated for potential risk, prior to application in agriculture and the environment by means of an independent review of potential risks on a case-by-case basis;
- Conduct the development of recombinant DNA organisms for agricultural and environmental applications in a stepwise fashion, moving, where appropriate, from the laboratory to the growth chamber and greenhouse, to limited field testing and finally to large-scale field testing; and
- Encourage further research to improve the prediction, evaluation, and monitoring of the outcome of applications of recombinant DNA organisms."

The role of confinement in small scale testing

In 1992, OECD published its Good Developmental Principles (GDP) (OECD, 1992) for the design of small-scale field research involving transgenic plants and micro-organisms. This document describes the use of *confinement* in field tests. Confinement includes measures, to avoid the dissemination or establishment of organisms from a field trial, for example, the use of physical, temporal, or biological isolation (such as the use of sterility).

Scale-up of crop-plants – "risk/safety analysis"

By 1993, the focus of attention had switched to the *scale-up* of crop plants as plant breeders began to move to larger-scale production and commercialisation of transgenic plants. OECD published general principles for, *scale-up* (OECD, 1993a), which re-affirmed that, "safety in biotechnology is achieved by the appropriate application of risk/safety analysis and risk management. Risk/safety analysis comprises

hazard identification and, if a hazard has been identified, risk assessment. Risk/safety analysis is based on the characteristics of the organism, the introduced trait, the environment into which the organism is introduced, the interaction between these, and the intended application. Risk/safety analysis is conducted prior to an intended action and is typically a routine component of research, development and testing of new organisms, whether performed in a laboratory or a field setting. Risk/safety analysis is a scientific procedure which does not imply or exclude regulatory oversight or imply that every case will necessarily be reviewed by a national or other authority" (OECD, 1993a).

The role of familiarity in risk/safety assessment

The issue of *scale-up* also led to an important concept, *familiarity*, which is one key approach that has been used subsequently to address the environmental safety of transgenic plants.

The concept of familiarity is based on the fact that most genetically engineered organisms are developed from organisms such as crop plants whose biology is well understood. It is not a risk/safety assessment in itself (U.S.-NAS, 1989). However, the concept facilitates risk/safety assessments, because to be familiar, means having enough information to be able to make a judgement of safety or risk (U.S.-NAS, 1989). Familiarity can also be used to indicate appropriate management practices including whether standard agricultural practices are adequate or whether other management practices are needed to manage the risk (OECD, 1993a). Familiarity allows the risk assessor to draw on previous knowledge and experience with the introduction of plants and micro-organisms into the environment and this indicates appropriate management practices. As familiarity depends also on the knowledge about the environment and its interaction with introduced organisms, the risk/safety assessment in one country may not be applicable in another country. However, as field tests are performed, information will accumulate about the organisms involved, and their interactions with a number of environments.

Familiarity comes from the knowledge and experience available for conducting a risk/safety analysis prior to scale-up of any new plant line or crop cultivar in a particular environment. For plants, for example, familiarity takes account of, but need not be restricted to, knowledge and experience with the following (OECD, 1993a):

- "The crop plant, including its flowering/reproductive characteristics, ecological requirements, and past breeding experiences;
- The agricultural and surrounding environment of the trial site;
- Specific trait(s) transferred to the plant line(s);
- Results from previous basic research including greenhouse/glasshouse and small-scale field research with the new plant line or with other plant lines having the same trait;
- The scale-up of lines of the plant crop varieties developed by more traditional techniques of plant breeding;
- The scale-up of other plant lines developed by the same technique;
- The presence of related (and sexually compatible) plants in the surrounding natural environment, and knowledge of the potential for gene transfer between crop plant and the relative; and
- Interactions between/among the crop plant, environment and trait.".

Risk/safety assessment and risk management

Risk/safety assessment involves the identification of potential environmental adverse effects or hazards, and determining, when a hazard is identified, the probability of it occurring. If a potential hazard or adverse affect is identified, measures may be taken to minimise or mitigate it. This is risk management. Absolute certainty or "zero risk" in a safety assessment is not achievable, so uncertainty is an inescapable aspect of all risk assessment and risk management (OECD, 1993a). For example, there is uncertainty in extrapolating the results of testing in one species to identify potential effects in another. Risk assessors and risk managers thus spend considerable effort to address uncertainty. Many of the activities in intergovernmental organisations, such as the OECD, address ways to handle uncertainty (OECD, 2000).

Appendix II

References cited in chronological order

OECD (1986), *Recombinant DNA Safety Considerations. Safety considerations for industrial, agricultural and environmental applications of organisms derived by recombinant DNA techniques* ("The Blue Book"), OECD, Paris.

U.S.-NAS (1989), *Field Testing of Genetically Modified Organisms: Framework for Decisions*, U.S. National Academy of Sciences, National Academy Press, Washington DC. USA.

OECD (1992), *Good Developmental Principles (GDP)*, OECD, Paris.

OECD (1993a), *Safety Considerations for Biotechnology: Scale-up of Crop Plants*, OECD, Paris.

OECD (1993b), *Traditional Crop Breeding Practices: An Historical Review to serve as a Baseline for Assessing the Role of Modern Biotechnology*, OECD, Paris.

OECD (1995a), *Commercialisation of Agricultural Products Derived through Modern Biotechnology: Survey Results*, OECD, Paris.

OECD (1995b), *Report of the OECD Workshop on the Commercialisation of Agricultural Products Derived through Modern Biotechnology*, OECD, Paris.

OECD (1995c), *Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology*, OECD, Paris.

OECD (1996), *Industrial Products of Modern Biotechnology Intended for Release to the Environment: the proceedings of the Fribourg Workshop*, OECD, Paris.

OECD (2000), *Report of the Working Group on the Harmonisation of Regulatory Oversight in Biotechnology to the G8 Okinawa Summit*, OECD, Paris.

Présentation des documents de consensus sur la sécurité biologique

1. A propos du Sous-groupe de l'OCDE pour la sécurité biologique

Le *Sous-groupe de l'OCDE sur l'harmonisation de la surveillance réglementaire en biotechnologie* (le Sous-groupe) comprend des délégués des 33 pays Membres de l'OCDE et de la Commission européenne. Généralement, les délégués sont des fonctionnaires des ministères et organismes gouvernementaux chargés de l'évaluation des risques pour l'environnement et de la sécurité des produits issus de la biotechnologie moderne. Le Sous-groupe comprend aussi plusieurs délégations et experts invités qui participent à ses travaux en qualité d'observateurs, notamment l'Argentine, la Fédération de Russie, le Programme des Nations Unies pour l'environnent (PNUE), le Secrétariat de la Convention sur la diversité biologique (SCDB), les Organisation des Nations Unies pour l'alimentation et l'agriculture (FAO) et pour le développement industriel (ONUDI) et le Comité consultatif économique et industriel auprès de l'OCDE (BIAC). Ces dernières années, du fait de l'utilisation croissante des produits issus de biotechnologie dans plusieurs régions du monde et le développement d'activités portant sur les espèces tropicales et sub-tropicales, la participation au Sous-groupe des économies non membres s'est intensifiée, avec notamment le Brésil, le Cameroun, la Chine, l'Estonie, l'Inde, les Philippines et l'Afrique du Sud.

2. Harmonisation de la réglementation

Le Sous-groupe a été créé en 1995[1] à l'époque des premières demandes d'autorisation réglementaire de cultures commerciales transgéniques dans plusieurs pays Membres de l'OCDE. Dès le départ, un des objectifs-clés du Sous-groupe a été de promouvoir l'harmonisation internationale de la réglementation en matière de biotechnologie entre les pays Membres. L'harmonisation réglementaire vise à assurer que les éléments d'information utilisés pour l'évaluation des risques et de la sécurité, ainsi que les méthodes pour les collecter, soient aussi uniformes que possible. Elle peut conduire les pays à reconnaître, voire à accepter les informations provenant d'évaluations réalisées par d'autres. Les avantages de l'harmonisation sont évidents. Elle accroît la compréhension mutuelle entre pays, ce qui évite la duplication des efforts, économise les ressources limitées et accroît l'efficacité des procédures d'évaluation des risques et de la sécurité. Tout cela a pour effet d'améliorer la sécurité, tout en réduisant les obstacles inutiles au commerce (OCDE, 2000).

3. Pourquoi mener des activités d'harmonisation à l'OCDE ?

La création du Sous-groupe et de son programme de travail résulte d'une réflexion approfondie menée par les pays Membres pour déterminer s'il convenait de poursuivre les travaux sur l'harmonisation en biotechnologie dans le cadre de l'OCDE et, dans l'affirmative, ce que ces travaux impliqueraient. Cette analyse a été conduite par le Groupe *ad hoc* sur les aspects environnementaux de la biotechnologie (créé par la Réunion conjointe[2]), principalement en1994.

Le Groupe *ad hoc* a pris en considération et mis à profit les précédents travaux menés à l'OCDEdès le milieu des années 80. Ces activités antérieures de l'OCDE étaient initialement centrées sur les conséquences pour l'environnement et l'agriculture des essais au champ d'organismes transgéniques, mais ont vite porté ensuite sur leur utilisation à grande échelle et leur commercialisation. (Un résumé de ces travaux figure en Appendice I.)

1 . Le Sous-groupe s'appelait à l'origine le « Groupe d'experts pour l'harmonisation de la surveillance réglementaire en biotechnologie ». Il est devenu un Sous-groupe de l'OCDE en 1998.

2 . La Réunion conjointe était l'organe de tutelle du Groupe *ad hoc* et qui a, au vu de ses résultats, établi le Sous-groupe comme un organe subsidiaire. Aujourd'hui, son nom officiel est la Réunion conjointe du Comité des produits chimiques et du Groupe de travail sur les produits chimiques, les pesticides et la biotechnologie.

4. Principaux concepts et principes fondamentaux

Le Groupe *ad hoc* a pris en compte les précédents travaux sur l'analyse des risques qui sont présentés dans le document intitulé *Considérations de Sécurité Relatives à la Biotechnologie : Passage à l'Échelle Supérieure des Plantes Cultivées* (OCDE 1993a). L'extrait suivant en donne un aperçu : « L'analyse de risque/de sécurité s'appuie sur les caractéristiques de l'organisme, le caractère introduit, l'environnement dans lequel l'organisme est libéré, les interactions de ces facteurs entre eux et l'utilisation prévue. » Ces travaux ont servi de point de départ à l'évaluation environnementale des risques et de la sécurité, aujourd'hui acceptée mondialement. En examinant les possibilités d'harmonisation, le Groupe *ad hoc* s'est intéressé à ces caractéristiques ainsi qu'aux informations utilisées par les évaluateurs des risques et de la sécurité pour les examiner.

A cela s'ajoute le concept de familiarité, aussi décrit dans le document mentionné ci-dessus (OCDE 1993a). Ce concept «… est basé sur le fait que la plupart des organismes transformés génétiquement sont développés à partir d'organismes comme les végétaux cultivés, dont la biologie est bien comprise ». « La familiarité permet à l'évaluateur de risques d'appliquer ses connaissances et son expérience de l'introduction des végétaux et des micro-organismes dans l'environnement. » S'agissant des végétaux, la familiarité tient compte d'un grand nombre d'éléments, par exemple, des connaissances et de l'expérience concernant « les végétaux cultivés, y compris leurs caractéristiques de floraison et de reproduction, leurs exigences écologiques et les expériences passées en matière de sélection » (OCDE 1993a – voir Appendice 1 pour une description plus détaillée). Cela montre bien le rôle des informations concernant la biologie de l'organisme hôte lors de l'évaluation des risques pour l'environnement et de la sécurité.

Le Groupe *ad hoc* a également pris en compte le document décrivant les «*Méthodes Traditionnelles de Sélection des Plantes : un Aperçu Historique Destiné à Servir de Référence pour l'Évaluation du Rôle de la Biotechnologie Moderne* » (OCDE 1993b), qui met l'accent sur les organismes hôtes. On y trouve des informations sur 17 espèces végétales cultivées qui sont (ou pourraient être) utilisées en biotechnologie moderne. Ce document comprend des sections consacrées aux aspects phytosanitaires du transfert de matériel génétique, et aux utilisations actuelles de ces plantes cultivées. Une section détaillée est également consacrée aux pratiques actuelles de sélection.

5. Une approche commune de l'évaluation des risques et de la sécurité

L'une des missions importantes du Groupe *ad hoc* était d'évaluer dans quelle mesure les pays Membres étudient les mêmes éléments et mêmes problèmes lors de l'évaluation des risques et de la sécurité. Des différences importantes indiqueraient des difficultés à rechercher une harmonisation ; à l'inverse, de nombreuses similitudes indiqueraient un travail d'harmonisation plus aisé.

Deux études ont permis au Groupe *ad hoc* de répondre à cette question. La première concernait les plantes cultivées (OCDE, 1995a ; 1995b) et la seconde les micro-organismes (OCDE, 1995c ; 1996). Ces deux études étaient basées sur une enquête auprès des autorités nationales chargées de l'évaluation des risques et de la sécurité. L'objectif était d'identifier les questions couvertes par le processus d'évaluation (selon la législation nationale, la réglementation ou les documents d'orientation) afin d'établir le degré de similitude entre les autorités nationales. Ces études ont pris pour référence les éléments contenu dans « *Le Livre bleu* » de l'OCDE sur les considérations de sécurité relatives à l'ADN recombiné (OCDE 1986), en particulier les sections relatives aux : i) Considérations scientifiques générales ; ii) Considérations relatives à la santé humaine ; et iii) Considérations relatives à l'environnement et l'agriculture (appendices b, c et d). Les deux études ont permis de constater que les questions et problèmes traités par les pays Membres pour évaluer les risques et la sécurité présentaient un remarquable degré de similitude.

6. Emergence du concept de « document de consensus »

Le Sous-groupe a donc été établi en sachant que les questions et problèmes traités par les autorités nationales aux fins de l'évaluation des risques et de la sécurité présentaient de nombreux points communs. Il a également pris en compte les caractéristiques identifiées dans le cadre de cette évaluation (*l'organisme*, *le caractère introduit*, et *l'environnement*), sur lesquels pouvaient se concentrer les activités d'harmonisation.

Il a été ensuite reconnu qu'une majeure partie des informations utilisées dans l'évaluation des risques et de la sécurité concernant la biologie des organismes hôtes (plantes cultivées, arbres, animaux ou micro-organismes), étaient identiques ou presque dans toutes les évaluations portant sur une même organisme. En d'autres termes, les questions relatives à la biologie de l'organisme examinées dans le cadre de l'évaluation des risques et de la sécurité --par exemple, le potentiel de transfert de gènes au sein d'une espèce cultivée, et entre espèces apparentées, de même que le caractère adventice potentiel-- sont les mêmes pour chaque demande impliquant une même espèce hôte. C'est aussi le cas, dans une certaine mesure, pour les informations relatives aux caractères introduits.

En conséquence, le Sous-groupe a souhaité regrouper les informations communes utilisées dans l'évaluation des risques et de la sécurité d'un certain nombre de produits transgéniques, et a décidé de se concentrer sur deux catégories particulières : la biologie des espèces hôtes ; et les caractères utilisés dans les modifications génétiques. L'objectif était d'encourager le partage de l'information et d'éviter la duplication d'efforts en permettant aux pays de ne pas traiter plusieurs fois des mêmes questions communes pour les demandes concernant le même organisme ou le même caractère. Il a été souligné que des documents de consensus sur la biologie ou sur les caractères pouvaient être adoptés relativement vite par les pays Membres (en quelques années). Ce processus de compilation a rapidement débouché sur la rédaction de documents de consensus.

7. Objet des documents de consensus

Les documents de consensus ne prétendent pas se substituer à l'évaluation des risques et de la sécurité, car ils ne concernent qu'une partie de l'information nécessaire. Cependant, ils devraient faciliter grandement l'évaluation environnementale des risques et de la sécurité.

Les documents de consensus visent à fournir un aperçu des données courantes pouvant être utilisées dans le processus d'évaluation réglementaire des produits issus de la biotechnologie. Ils ne prétendent pas offrir une source d'informations exhaustive sur l'ensemble des connaissances concernant un organisme hôte ou un caractère particulier ; mais ils traitent, sur la base d'un consensus, des questions centrales jugées pertinentes par les pays pour l'évaluation des risques et de la sécurité.

Ces documents visent à faciliter l'échange d'informations sur ces composantes clés des évaluations de la sécurité environnementale afin d'éviter que les activités menées dans les pays ne fassent double emploi. Ils sont destinés : a) aux demandeurs d'autorisation pour indiquer le type d'information à fournir aux autorités de réglementation ; b) aux autorités chargées de la réglementation comme guide général et source de référence pour leurs examens ; et c) aux gouvernements aux fins de l'échange d'information, comme références de recherche et pour l'information du public.

Il a été déclaré initialement que les informations contenues dans les documents de consensus devaient être *mutuellement reconnues ou mutuellement acceptées* par les pays Membres de l'OCDE, bien que le sens de ces expressions reste encore à préciser. L'expression *acceptation mutuelle des données* a été étudiée pendant la période de mandat du Groupe *ad hoc* et le début du Sous-groupe (1993-1995). Cette notion est empruntée au Programme des produits chimiques de l'OCDE pour désigner un ensemble de Décisions du Conseil de l'OCDE qui ont un caractère contraignant pour les pays Membres. Dans le cas des documents de consensus, il n'a jamais été obligatoire d'utiliser les informations y figurant, même si le Sous-groupe est intéressé à impliquer davantage les pays dans l'utilisation de ces documents.

La participation des pays à l'élaboration des documents, et leur intention d'utiliser les informations qu'ils contiennent, sont présumées de bonne foi. On attend donc des documents de consensus qu'ils puissent servir de référence dans les évaluations des risques et de la sécurité. Ces documents étant des outils d'accès public, ils peuvent intéresser tout pays qui souhaite les utiliser dans des évaluations nationales.

8. Processus de développement des documents de consensus jusqu'à publication

La rédaction d'un document de consensus se fait en plusieurs étapes. Cela commence lorsqu'une délégation, à l'occasion d'une réunion officielle du Sous-groupe, propose d'établir un document sur un nouveau sujet, le plus souvent une espèce cultivée ou un caractère. Si le Sous-groupe approuve la proposition, un premier projet est préparé par un, ou deux ou plusieurs pays en collaboration (« approche par pays pilote »). En général, le ou les pays pilote(s) possèdent une expérience de la plante ou du caractère visé et peuvent identifier des experts pour préparer une première version.

Cette version préliminaire est d'abord examinée par le Bureau du Sous-groupe[3] qui vérifie que le document couvre bien tous les aspects généralement pris en compte par les documents de consensus, et que sa qualité est suffisante pour le soumettre à l'ensemble du Sous-groupe.

Un premier projet est établi à la lumière des commentaires du Bureau, puis présenté à l'ensemble du Sous-groupe. Chaque délégation peut ainsi étudier le texte et formuler des commentaires en fonction de l'expérience de son pays. Ces commentaires sont pris en compte dans une deuxième version qui est à nouveau diffusée au Sous-groupe. A ce stade, le Sous-groupe peut être invité à recommander la déclassification du document. Cette demande intervient uniquement lorsque toutes les délégations ont décidé d'un commun accord (consensus) que le document était complet et prêt pour publication. Il arrive cependant que le texte nécessite un troisième examen, voire plus, par le Sous-groupe avant qu'une déclassification ne puisse être envisagée.

Lorsque le Sous-groupe est convenu de recommander le document pour déclassification, il est transmis à l'organe de tutelle, la Réunion conjointe, qui est invitée à le déclassifier. Une fois approuvé par la Réunion conjointe, le document est publié.

Il importe de noter que l'examen des documents de consensus dépasse le cadre des réunions officielles du Sous-groupe. De nombreux échanges de vues se font aussi par voie électronique, notamment dans le cadre du site Web protégé dédié au Sous-groupe. Ceci permet à divers experts de compléter les projets.

Pour plusieurs des documents, il s'est révélé nécessaire d'inclure aussi des informations provenant de pays non membres. Cet échange élargi d'expertise est devenu de plus en plus important ces dernières années avec le développement d'activités portant sur les espèces tropicales et sub-tropicales. Cela s'est produit notamment pour les plantes cultivées dont les centres d'origine et de diversité se trouvent dans un ou des pays non membre(s). Le PNUE, l'ONUDI et la FAO ont alors contribué à la préparation des documents en identifiant les experts appropriés des pays concernés. C'était le cas, par exemple, lors de l'élaboration du document de consensus sur la biologie du riz.

9. Évolutions actuelles et futures pour le Sous-groupe

Le Sous-groupe poursuit ses travaux de préparation des documents de consensus, ainsi que l'amélioration de l'efficacité de leur processus d'élaboration. Un nombre croissant de plantes cultivées et autres espèces hôtes (arbres, animaux, micro-organismes) fait l'objet de modifications, pour un nombre

3 . Le Bureau comprend le Président et les Vice-présidents du Sous-groupe. Le Bureau est élu par le Sous-groupe une fois par an. Au moment où de la préparation de la présente publication - Vol. 3 et Vol. 4, le Président est un délégué des Etats-Unis, les Vice-présidents sont du Canada, de la Finlande, du Japon et des Pays-Bas.

croissant de caractères transférés, et le Sous-groupe vise à satisfaire les besoins actuels tout en préparant les sujets émergeants.

Lors de l'atelier de l'OCDE consacré aux documents de consensus et aux travaux futurs d'harmonisation, tenu à Washington D.C. en octobre 2003, le Sous-groupe a examiné comment établir un ordre de priorité pour la préparation des documents de consensus parmi les nombreux classements possibles. Les participants ont également reconnu qu'il pourrait être nécessaire de périodiquement revoir ou de mettre à jour les documents de consensus, pour veiller à ce qu'ils contiennent les informations les plus récentes. Le Sous-groupe examine régulièrement ces aspects lorsqu'il planifie son futur travail. Pour faciliter l'élaboration des prochains documents, les participants à l'atelier ont jugé utile d'établir une structure type de document de consensus. Le Sous-groupe a donc décidé de préparer des documents guides listant les « points à examiner », tout d'abord pour les documents de consensus relatifs à la biologie, puis pour ceux portant sur les caractères. Le texte sur les documents de biologie, publié en 2006, figure en section suivante de la présente publication.

10. Le Groupe d'étude de l'OCDE sur la sécurité des nouveaux aliments destinés à la consommation humaine et animale

Le *Groupe d'étude de l'OCDE sur la sécurité des nouveaux aliments destinés à la consommation humaine et animale* (Groupe d'Etude), établi en1999, traite des aspects de l'évaluation des aliments issus des cultures transformées génétiquement et destinés à la consommation humaine et animale. L'objet principal du travail du Groupe d'étude, similaire à celui du Sous-groupe, est d'assurer que les types d'information utilisés lors de l'évaluation des risques et de la sécurité, ainsi que les méthodes utilisées pour collecter cette information, sont aussi similaires que possibles entre pays. L'approche consiste à comparer les cultures transgéniques et les produits qui en dérivent avec leurs homologues conventionnels qui sont déjà connus et considérés comme sûrs du fait de l'expérience reconnue de leur utilisation. Les activités du Groupe d'étude facilitent les méthodes harmonisées et un échange d'information.

Comme pour le programme sur la sécurité biologique, le produit principal du programme sur les aliments est la Série de documents de consensus sur la composition des nouvelles variétés d'espèces cultivées. Les documents du Groupe d'étude compilent une base commune d'information scientifique sur les composants essentiels des plantes cultivées tels que les principaux nutriments, toxiques, anti-nutriments et allergènes. Ces documents constituent des outils pratiques pour les responsables de la réglementation et les autorités en charge de l'évaluation des risques et de la sécurité de ces nouvelles variétés, en ce qui concerne les aliments destinés à la consommation humaine et animale. A ce jour, 20 documents de consensus on été publiés sur les espèces cultivées majeures, et sur des aspects généraux visant à faciliter l'harmonisation. Ils constitutent la Série sur la Sécurité des Nouveaux Aliments Destinés à l'Alimentation Humaine et Animale, également accessible gratuitement en ligne sur le site de l'OCDE (www.oecd.org/biotrack).

La publication de l'intégralité de la Série sur les Nouveaux Aliments, rassemblée en un seul document, est envisagée au cours de l'année 2011.

Le Sous-groupe et le Groupe d'étude mettent en oeuvre des programmes à la fois proches et complémentaires, centré sur les aspects environnementaux pour le premier, sur les aspects d'alimentation humaine et animale pour le second. Leur coopération sur des sujets d'intérêt commun a résulté récemment dans la publication du premier document de consensus élaboré conjointement par les deux instances, intitulé « *Consensus Document on Molecular Characterisation of Plants Derived from Modern Biotechnology* » (*Document de Consensus sur la Caractérisation Moléculaire des Plantes issues de la Biotechnologie Moderne*)", édité en septembre 2010. Ce document est reproduit dans le Volume III de la présente publication.

Appendice I

Principes et concepts de l'OCDE relatifs à la sécurité biologique, établis antérieurement à la création du Sous-groupe (1986-1994)

Depuis le milieu des années 1980, l'OCDE a développé des approches harmonisées pour l'évaluation des risques et de la sécurité des produits de biotechnologie moderne. Avant la création du Sous-groupe, l'OCDE a publié plusieurs rapports d'experts portant sur des questions de sécurité, les concepts et principes relatifs à l'évaluation des risques et de la sécurité, ainsi que sur la dissémination des plantes transgéniques en champ, et sur la question des pratiques traditionnelles de sélection des espèces cultivées. Le présent appendice récapitule les éléments essentiels de ces travaux qui ont servi de point de départ à la création du Sous-groupe et à l'élaboration des documents de consensus.

Principes scientifiques sous-jacents

En 1986, l'OCDE a publié ses premières études sur la sécurité des organismes transgéniques (OCDE, 1986). Celles-ci comprenaient des questions intéressant la santé humaine, l'environnement et l'agriculture qui pourraient être prises en compte dans l'évaluation des risques et de la sécurité. Dans les recommandations pour les applications agricoles et environnementales, il était suggéré que les évaluateurs des risques et de la sécurité :

- « Utilisent des données nombreuses sur les effets au niveau de l'environnement et de la santé humaine des organismes vivants afin de guider les évaluations des risques ;

- Assurent que les organismes formés de molécules d'ADN recombiné sont évalués pour déterminer les risques possibles, préalablement à leur application dans l'agriculture et dans l'environnement par un examen distinct des risques potentiels de façon ponctuelle ;

- Dirigent le développement d'organismes formés d'ADN recombiné pour des applications agricoles et environnementales d'une manière progressive, allant si approprié, du laboratoire à la chambre de culture et à la serre, puis à des essais limités en conditions réelles, et finalement à des essais au champ à grande échelle ;

- Encouragent la recherche pour améliorer les prédictions, l'évaluation et le suivi des résultats des applications d'organismes formés d'ADN recombiné. »

Rôle du confinement dans les essais à échelle réduite

En 1992, l'OCDE a publié ses Bons Principes de Developpement (OCDE, 1992) pour la conception de recherche sur le terrain à échelle réduite impliquant des végétaux et micro-organismes transgéniques. Ce document décrit l'utilisation du *confinement* des essais de terrain. Le confinement comprend des mesures pour éviter la dissémination ou l'établissement d'organismes à partir d'un essai de terrain, par exemple les mesures d'isolement physique, temporel ou biologique (comme l'utilisation de la stérilité).

Mise à l'échelle des végétaux cultivés – « analyse des risques et de la sécurité »

À partir de 1993, l'attention s'est dirigée vers la *mise à l'échelle* des végétaux cultivés du fait que les sélectionneurs commençaient à envisager la production à grande échelle et la commercialisation de plantes transformées génétiquement. L'OCDE a publié les principes généraux pour la *mise à l'échelle* (OCDE,

1993a), lesquels réaffirmaient que, « La sécurité en biotechnologie est réalisée par l'application appropriée de l'analyse des risques et de la sécurité et de la gestion des risques. L'analyse des risques et de la sécurité comprend l'identification des dangers et, si un danger a été identifié, la gestion du risque. L'analyse des risques et de la sécurité est fondée sur les caractéristiques de l'organisme, le trait caractéristique introduit, l'environnement dans lequel l'organisme est introduit, les interactions entre l'environnement et l'organisme de même que l'application prévue. L'analyse des risques et de la sécurité est menée préalablement à une action visée et est en général une composante de routine de la recherche, du développement et des essais de nouveaux organismes, que ces actions soient effectuées en laboratoire ou sur le terrain. L'analyse des risques et de la sécurité est une procédure scientifique qui n'implique ni n'exclut une surveillance au niveau de la réglementation, et qui n'exige pas que chaque cas soit nécessairement examiné par une autorité nationale ou autre » (OCDE, 1993a).

Rôle de la familiarité dans l'évaluation des risques et de la sécurité

La question de la *mise à l'échelle* a également mené à un concept important, la *familiarité*, qui est l'une des approches stratégiques utilisées par la suite pour traiter de la sécurité environnementale des végétaux transgéniques.

Le concept de familiarité est basé sur le fait que la plupart des organismes transformés génétiquement sont développés à partir d'organismes comme les plantes cultivées dont la biologie est bien comprise. Il ne constitue pas une évaluation des risques et de la sécurité en tant que tel (U.S.-NAS, 1989). Toutefois, le concept facilite les évaluations des risques et de la sécurité parce que la familiarité suppose que l'on dispose de suffisamment d'éléments pour être en mesure de poser un jugement sur la sécurité ou sur le risque (U.S.-NAS, 1989). La familiarité peut aussi servir à identifier les pratiques de gestion appropriées, comme par exemple déterminer si les pratiques agricoles usuelles sont adéquates ou si d'autres conduites des cultures sont nécessaires pour gérer le risque (OCDE, 1993a). La familiarité permet à l'évaluateur de risques d'appliquer ses connaissances et son expérience de l'introduction des végétaux et des micro-organismes dans l'environnement, ce qui lui indique les pratiques de gestion appropriées. Comme la familiarité dépend aussi de la connaissance de l'environnement et de ses interactions avec les organismes introduits, l'évaluation des risques et de la sécurité effectuée dans un pays peut ne pas s'appliquer à un autre pays, Toutefois, au fur et à mesure des essais en champ, l'information grandit sur les organismes impliqués et sur leurs interactions avec divers environnements.

La familiarité provient des connaissances et de l'expérience disponibles pour analyser les risques et la sécurité préalablement à la mise à l'échelle de toute nouvelle lignée végétale ou variété cultivée dans un environnement particulier. Pour les végétaux par exemple, la familiarité tient compte, sans y être limitée, des connaissances et de l'expérience au niveau (OCDE, 1993a) :

- « des végétaux cultivés, y compris leurs caractéristiques de floraison et de reproduction, leurs exigences écologiques et les expériences passées en matière de sélection des végétaux ;
- de l'environnement agricole et environnant du site d'essais ;
- du ou des trait(s) caractéristique(s) spécifique(s) transféré(s) à la ou les lignée(s) de végétaux ;
- des résultats des précédents travaux de recherche fondamentale, notamment la recherche en serre et à échelle réduite sur la nouvelle lignée de végétaux ou sur d'autres lignées présentant les mêmes traits caractéristiques ;
- de la mise à l'échelle de lignées de végétaux cultivés développés par des techniques plus traditionnelles de sélection des végétaux ;

- de la mise à l'échelle d'autres lignées de végétaux développées par la même technique ;

- de la présence de végétaux apparentés (et sexuellement compatibles) dans l'environnement naturel et des connaissances au niveau de la possibilité de transfert génique entre la plante cultivée et la plante apparenté ;

- des interactions entre la plante cultivée, l'environnement et les traits caractéristiques et des interactions au sein de la plante cultivée. »

Évaluation des risques et de la sécurité, et gestion des risques

L'évaluation des risques et de la sécurité suppose l'identification des effets nocifs ou des dangers possibles pour l'environnement et la détermination, lorsqu'un danger est identifié, de la probabilité qu'il se produise. Si un danger ou un effet nocif sur la santé est identifié, des mesures doivent être entreprises pour le minimiser ou l'atténuer. C'est ce que l'on appelle la gestion des risques. La certitude absolue, ou l'absence totale de risques, est impossible à obtenir en matière d'évaluation de la sécurité. L'incertitude est donc un aspect inévitable de toutes les évaluations des risques et de toute gestion des risques (OCDE, 1993a). Par exemple, l'on retrouve de l'incertitude en extrapolant les résultats des tests effectués sur une espèce pour identifier les effets possibles chez une autre espèce. Les évaluateurs et les gestionnaires de risques déploient donc des efforts considérables à traiter les incertitudes. Plusieurs des activités des organisations gouvernementales, comme l'OCDE, tentent de déterminer des façons de gérer ces incertitudes (OCDE, 2000).

Appendice II

Références (par ordre chronologique)

OCDE (1986), *Considérations de sécurité relatives à l'ADN recombiné. Considérations de sécurité relatives à l'utilisation d'organismes obtenus par des techniques de recombinaison de l'ADN dans l'industrie, dans l'agriculture et dans l'environnement* («Le Livre Bleu »), OCDE, Paris.

U.S.-NAS (1989), *Field Testing of Genetically Modified Organisms: Framework for Decisions*. U.S. National Academy of Sciences, National Academy Press, Washington D.C., États-Unis.

OCDE (1992), *Bons Principes de Développement (BPD)*, OCDE, Paris.

OCDE (1993a), *Considérations de sécurité relatives à la biotechnologie : Passage à l'échelle supérieure des plantes cultivées*, OCDE, Paris.

OCDE (1993b), *Méthodes traditionnelles de sélection des plantes : Un aperçu historique destiné à servir de référence pour l'évaluation du rôle de la biotechnologie moderne*, OCDE, Paris.

OCDE (1995a), *Commercialisation of Agricultural Products Derived through Modern Biotechnology: Survey Results*, OCDE, Paris.

OCDE (1995b), *Report of the OECD Workshop on the Commercialisation of Agricultural Products Derived through Modern Biotechnology*, OCDE, Paris.

OCDE (1995c), *Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology*, OCDE, Paris.

OCDE (1996), *Industrial Products of Modern Biotechnology Intended for Release to the Environment: the Proceedings of the Fribourg Workshop*, OCDE, Paris.

OCDE (2000), *Rapport du Sous-groupe sur l'harmonisation de la surveillance réglementaire en biotechnologie au Sommet du G8 d'Okinawa*, OCDE, Paris.

Points to consider for consensus documents on the biology of cultivated plants

Introduction

Most environmental risk/safety assessments of transformed (genetically modified or engineered) plants are based upon a broad body of knowledge and experience with the untransformed species based on familiarity with the crop plant. The intent of the biology consensus documents is to describe portions of this body of knowledge directly relevant to risk/safety assessment in a format readily accessible to regulators. The document is not an environmental risk/safety assessment of the species. Rather, the consensus document provides an overview of pertinent biological information on the untransformed species to help define the baseline and scope (the comparator against which transformed organisms will be compared), in the risk/safety assessment of the transformed organism. Consensus documents are not detailed crop handbooks or manuals of agricultural or silvicultural practice or economic botany, but rather focus on the biological information and data that may be clearly relevant to the assessment of newly transformed plants.

This *Points to Consider* document is meant as a structured explanatory checklist, regarding both order and contents, of relevant points to consider in preparing or evaluating a consensus document on the biology of a cultivated vascular plant species or other taxonomic group of interest, in relation to biotechnology and environmental risk/safety assessment. The general approach laid out in this document may also be pertinent to non-vascular plants (for example mosses) as well as fungi and micro-organisms; however, these groups are biologically and ecologically so different that further adaptation and refinement of the general approach will be necessary.

The biology consensus documents that have been published to date, as well as most in preparation, deal with crops, timber trees, and fruit trees [excepting the one on *Pleurotus* spp. (oyster mushrooms) and several on micro-organisms]. The plants of interest that have been the subject of the documents are primarily row crops, or trees managed silviculturally or grown in plantations or orchards. They are vascular plants, either flowering plants (angiosperms) or conifers (gymnosperms).

The points to consider as covered in the present document create a basic format and scope to be used for writing or reviewing new consensus documents and updating the earlier documents. While this *Points to Consider* document is meant to provide a basic format and scope, it is not intended to be rigid or inflexible. Of the biology consensus documents to date, some have addressed a particular point in depth, others lightly, and some not at all, depending on the relevance of the point to the plant species or other group of interest. Should additional points beyond those covered in this document be needed for a particular plant, the additional information can be included in the body of the consensus document, or in appendices. If a particular point is not covered in a consensus document, the text may briefly explain why the point, in the particular case, is not relevant.

Authors of the draft of a plant biology consensus document should be familiar with this *Points to Consider* document as well as existing consensus documents in the OECD Series on Harmonisation of Regulatory Oversight in Biotechnology (SHROB), in order to develop the appropriate scoping and presentation of information and data and for general editorial style. Existing consensus documents, particularly more recent ones, may provide detailed examples (some noted below) that are helpful models or thought-provoking for particular cases.

An understanding of the biology of the species or other group of interest will aid in determining the kinds of information pertinent to the environmental risk/safety assessment. This *Points to Consider* document provides an explanation of why the point (as enumerated below) is important in risk/safety assessment of the transformed plant, and presents a rationale for how the information in the point relates to risk/safety assessment. For a particular environmental risk/safety assessment, biological or ecological information in addition to that presented in the consensus document may be needed to address the regional environments into which the genetically engineered plant is proposed to be released.

1. Species or taxonomic group

The focus of each biology consensus document has usually been a species, but in some cases the focus has been a group of species or a genus, or just a subspecies or a cultivar group (examples are below). The primary focus of this *Points to Consider* document also is the species of interest, so appropriate adjustments will be necessary if the focus of the consensus document is more broad or narrow.

1.1. Classification and nomenclature

Give the scientific name of the cultivated species of interest, with its authors, and pertinent synonyms (*i.e*. actively used alternative scientific names, if any). If necessary to delimit the plant, also give the horticultural name, *e.g.* the cultivar group (*e.g. Beta vulgaris* subsp. *vulgaris* Sugar Beet Group). Provide main international common name(s) at least in English for the species of interest. Give the taxonomic context of the species (family always, perhaps the order, and perhaps the subfamily, tribe, subgenus or section). If the taxonomy is not settled, be relatively conservative in choosing the taxonomy, and briefly explain the alternative(s). The latest taxonomic or nomenclatural study is not necessarily definitive, and may need time for scientific consensus before it becomes adopted. A common name for the crop species of interest can be introduced here, to be used in much of the document as a more familiar name (aide-memoire).

Describe the taxonomic relationships of the cultivated species: related species, and related genera particularly if there is good potential for spontaneous hybridisation or the generic limits are unsettled. A list of related species (with brief ranges) should be given and include all the relatives with a potential for hybidization (*i.e.* crossable relatives). This topic is dealt with in detail in Section IV. The listing here may provide brief information on chromosome numbers and ploidy if these data are pertinent to the taxonomic differentiation of the species, whereas a more complete coverage of the relevant details is provided in Section III or IV.

> ***Rationale***: The scientific name enables an unequivocal understanding (*i.e.* a circumscription) of the plant of interest, at the appropriate level, such as the species or the subspecies. This addresses what the species (or other group) is and what it is called (*i.e.* circumscription and name). The list of close relatives could help in subsequent analysis to form an idea of the kinds of pertinent traits such as disease resistance or stress tolerance that may already occur in these direct relatives of the cultivated plant, and may help elucidate how genes/traits are shared and may move *via* gene flow amongst related populations. The list of close relatives may aid in understanding the range of diversity and variability between the species and its natually crossable relatives.
>
> ***Examples***: OECD Series on Harmonisation of Regulatory Oversight in Biotechnology (SHROB) No. 14 (rice, Section II, pp. 12-14); No. 16 (poplars, Section II, pp. 15-18); No. 18 (sugar beet, Section I, pp. 11-12); No. 22 (eastern white pine, Section II, p. 12); and No. 31 (sunflower, Section I, pp. 11-13).

1.2. Description

Give a brief non-technical description of the species of interest, understandable to the non-specialist. Provide the habit and general characteristics of the plant, for example that it is an annual, a long-lived tree, or a biennial cultivated as an annual crop, and that it is, for instance, grown for fibre, fruit, or seeds. Also provide a concise technical (taxonomic) description sufficient to make a positive identification of the plant (or part). Illustration (a line drawing or black-and-white photo) may be useful. To clarify distinctiveness, emphasise the practical diagnostic or distinguishing morphological or other characters. Limit jargon, by the precise use of phrases and familiar words. A table of main differences or taxonomic

key may be instructive (*e.g. Oryza sativa* and *O. glaberrima* in SHROB No. 14). If necessary, for example when based on recent information or a new approach, present or reference the analytical methods by which a differential identification of the similar plants (*e.g.* species) is now made.

> ***Rationale***: These descriptions provide broad orientation, and as well accurate identification. They briefly explain how the species of interest is actually identified in relation to others. Additionally, the description may give particular characteristics of the plant to aid in defining the scope of a risk/safety assessment. Although an exact identification often is based on experience (*i.e.* recognition) or on regional publications, rigorous or subtle analysis using specialist resources sometimes is required.
>
> ***Examples***: OECD SHROB No. 8 (potato, Section IV, pp. 14-15) and No. 28 (European white birch, Section I, pp. 12-13).

1.3. Geographic distribution, natural and managed ecosystems and habitats, cultivation and management practices, and centres of origin and diversity

This subsection covers the primary or crop species of interest, including the plants that are wild or free-living (whether native or naturalised) or weedy, and as cultivated or managed in the field. Crossable relatives with the relevant information and data on their intraspecific and interspecific crossing are discussed in Sections III and IV.

1.3.1. Geographic distribution

Describe the overall geographic distribution (if helpful including altitudinal range or climatic region), indicating broadly where the species of interest is native (*i.e.* indigenous), where it has been naturalised (introduced but free-living), and where it is in cultivation. A general map may be useful.

> ***Rationale***: Knowledge of the geographic distribution sets the context for understanding the potential interaction of the species with its relatives and with the surrounding ecosystems. For example, it is important to make a distinction between the species' native and naturalised occurrence when assessing the potential effects and the importance of gene flow.
>
> ***Examples***: OECD SHROB No. 8 (potato, Sections II & III, pp. 12-13); No. 13 (white spruce, Section III, pp. 15-16); and No. 16 (poplars, Section II, pp. 15-18).

1.3.2. Ecosystems and habitats where the species occurs natively, and where it has naturalised

Indicate the natural and non-cultivated or non-managed ecosystems where populations of the species of interest are native (indigenous) and where introduced and now naturalised (free-living) components of the vegetation. Designated natural areas (*e.g.* protected reserves, parks) where the species may be an invasive problem would be noted here. A species weedy in disturbed waste (*e.g.* abandoned) areas would be included here, whereas the species weedy in intensively managed areas would be discussed in the following subsection. Those ecosystems and habitats in which the species of interest occurs and its abundance are indicated here, whereas its ecological interactions with biotic components of the ecosystems and habitats are developed in Section V.

> ***Rationale***: The focus of this subsection is the relatively natural, self-sustaining context, rather than the land areas strongly managed for plant production. Knowledge of where the species occurs indigenously or is free-living provides baseline information for understanding the range of habitats in which the species exists, the range of behaviours exhibited in those habitats, and how characteristics of the species determine the range of habitats where it occurs. This information provides an understanding of the species' potential for interaction with its relatives and surrounding habitats.

Example: OECD SHROB No. 28 (European white birch, Section III, pp. 19-20).

1.3.3. Agronomic, silvicultural, and other intensively managed ecosystems where the species is grown or occurs on its own, including management practices

Describe where the species is dependent on management for survival or persistence over several years of usual conditions. Areas where the plant may be a weed problem would be discussed here. Areas to be discussed could include habitats such as annual row crops or bordering areas, tree plantations, orchards and vineyards, along regularly managed roadsides, rights-of-way, irrigation ditches, etc. Identify the pertinent general agronomic or other practices, and if relevant, regional differences in practices (including various practices within a region). Information might briefly encompass site preparation after clear-cutting, tillage, sowing or planting, weed control, control of volunteers, harvesting, plant protection practices during crop growth and after harvest, transport practices, and the use of harvested materials (*e.g.* for silage). The relevant ecological interactions of the species with particular organisms in these managed ecosystems are discussed in Section V.

Rationale: The focus of this subsection is on the plant's survival in agro-ecological, silvicultural, and other such managed areas, to provide the baseline environmental information on how the plant responds to or is managed by accepted agronomic, silvicultural or similar intensive practices. Identification of significant cultivation or management practices provides an understanding of measures available to manage or control the plant.

Examples: OECD SHROB No. 7 (oilseed rape, Section III, p. 13); No. 14 (rice, Section VII, pp. 26-27); No. 15 (soybean, Sections II & V, pp. 13 & 14); and No. 18 (sugar beet, Sections I & II, pp. 16-17).

1.3.4. Centres of origin and diversity

Describe the known or probable primary centre(s) of origin, as well as secondary centres where additional important variability or biodiversity may occur, whether naturally (*e.g. Beta*) or through the process of domestication (*e.g. Zea mays*, *Solanum tuberosum* subsp. *tuberosum*). The evolutionary centres important for natural biodiversity should be mentioned, and the central areas of domestication and landrace diversity, with indication of the centres' relative importance. Genetic diversity is covered in Section III. Provide a brief sketch of the history or extent of domestication including mention of relevant domestication traits (*e.g.* non-shattering, loss of seed dormancy).

Rationale: The interaction of the cultivated plant with close relatives especially in a centre of origin is an important consideration because gene flow, varietal competition, or a change in cultivation practices may alter this especially rich and valuable diversity. If the plant is not expected to be grown near a center of diversity, the absence of such relatives would also be important. A brief review of domestication may provide insight showing the continuity of modification of the species and the degree of the crop plant's adaptation to or dependence on the managed environment.

Examples: OECD SHROB No. 9 (bread wheat, Section III, pp. 13-16); No. 27 (maize, Section IV, pp. 18-20); and No. 31 (sunflower, Section I, pp. 14-15.

2. Reproductive biology

2.1. Generation time and duration under natural circumstances, and where grown or managed

Important aspects of generation time and duration include the time to first flowering and total life cycle of the plant, and time from planting to plow-down. Include the effects of agronomic, silvicultural, and similar practices when describing generation time and duration of the cultivated plant. Important differences within both the natural and the cultivated regions should be noted.

> ***Rationale***: The generation time and duration are indications of the terms in which environmental effects may occur. Precocious generation times and shorter durations in agriculture affect the likelihood of outcrossing with free-living (wild) relatives, and give a general indication of when outcrossing may first occur.
>
> ***Examples***: OECD SHROB No. 14 (rice, Sections V & VII, pp. 21 & 26-27) and No. 18 (sugar beet, Section I, pp. 13-14).

2.2. Reproduction (production of flowers or cones, fruits, seeds, and vegetative propagules)

Include a characterisation of the key stages in the life cycle necessary for the plant to survive, reproduce, and disperse. Particular attention is given to any uncommon survival structures or strategies and their importance under natural and cultivation conditions, and to the dependence of survival and reproduction on ecological and geographical factors.

> ***Rationale***: The reproductive capabilities of a plant determine the means by which the plant can produce progeny and spread or disperse. Both the plant and its progeny may affect the environment, including other organisms, and thus the time frame and geographic area over which effects might occur.

2.2.1. Floral biology

Describe the general floral dynamics (*e.g*. flowering season, flowering time, anthesis, selfing and/or outcrossing). Relevant genetic details of the outcrossing and/or selfing are addressed in Section III.

> ***Rationale***: This information will assist in understanding some of the factors that affect the potential for gene flow, and in assessing particular management strategies for reducing gene flow when outcrossing may occur. Such management strategies may include induced male sterility or asynchronous flowering times.
>
> ***Examples***: OECD SHROB No. 8 (potato, Section VI, p. 17); No. 14 (rice, Section V, p. 21); and No. 21 (Sitka spruce, Section III, p. 15).

2.2.2. Pollination (wind, insects, both, etc.), pollen dispersal, pollen viability

Describe observed modes of pollen dispersal, indicating the most prevalent way. Important insect or other animal pollinators should be indicated. Give data on the range of pollen dispersal through the air and/or by the animal vectors, if known. Note how climatic or regional (*e.g.* geographic) differences can affect pollination. Provide available information or data on the influence of pollen quantity, movement, viability, load and competition on outcrossing, which is discussed in Sections III and IV. The details on pollination as they pertain to the plant are covered here, whereas details particularly pertinent to the pollinator are covered in Section V.

Rationale: Pollen biology is an important component in the assessment of potential for gene flow, and in the evaluation of a need for and the type(s) of pollen confinement strategies such as buffer rows or isolation distances.

Examples: OECD SHROB No. 8 (potato, Section VI, p. 17) and No. 18 (sugar beet, Section IV, pp. 22-23).

2.2.3. Seed production, and natural dispersal of fruits, cones, and/or seeds

Briefly describe the sexual reproductive structures, including relevant morphological characteristics of fruits (or cones) and seeds, and note any inherent means of dispersal (*e.g.* shattering, fruit splitting, ballistic). Note the quantity of seeds produced by a plant (*e.g.* seeds per fruit and number of fruits). Provide information on the means and range of dispersal (*e.g.* by gravity, wind, water, on and/or in animals), and if there are several means indicate their relative importance. Cover apomixis below, in Subsection 2.2.5.

Rationale: The number of seeds and seed/fruit dispersal mechanisms are factors to consider in understanding the potential for establishment of free-living plants or populations, and thus the time and geographic area over which environmental effects might occur. The range of variability of these factors is also an important consideration.

Examples: OECD SHROB No. 15 (soybean, Section IV, p. 14) and No. 28 (European white birch, Section IV, p. 23).

2.2.4. Seed viability, longevity and dormancy, natural seed bank; germination, and seedling viability and establishment

Discuss factors in the establishment of any seed bank, including its transience or persistence, and the viability, longevity and dormancy of seeds under natural conditions. Note any special conditions that affect dormancy and/or germination (*e.g.* depth of burial, light and/or temperature, passage through an animal's digestive tract, or need for fire) that might be particularly relevant. Note any special requirements for the establishment and survival of seedlings (*e.g.* soil qualities or regime), as the organism's fitness may be revealed at this challenging phase in the life cycle.

Rationale: Seed viability is a key factor to consider in assessing the likelihood of survival of non-cultivated plants. Natural seed banks are often the main source of weeds in cultivated fields, whether they are previous-crop volunteers or non-crop weedy relatives. Whether seedlings can establish usually is a primary limiting factor in continuing the life cycle.

Example: OECD SHROB No. 7 (oilseed rape, Section VI, p. 17).

2.2.5. Asexual propagation (apomixis, vegetative reproduction)

Take into account natural vegetative cloning (*e.g.* in grasses and poplars), the kinds of propagules (special structures, and/or fragmented plant pieces), dispersal of the propagules, and their viability. Discuss the relative importance of asexual reproduction for the plant, including any differences dependent on habitat or region. For apomixis (non-sexual production of seeds), similarly consider its relative importance and effectiveness.

Rationale: If a plant has a strategy that includes asexual propagation, this could be a means for considerable or quite different dispersal or spread, and consequently may also affect the time frame and geographic area over which environmental effects might occur.

Example: OECD SHROB No. 16 (poplars, Section IV, p. 23).

3. Genetics

3.1. Relevant detailed genetic information on the species

Give a basic overview of the relevant genetic constitution and genetic dynamics of the species. If more appropriate in a particular case, some basic genetic information (*e.g.* ploidy, ancestral/progenitor genomes) may be more fully or instead discussed in Section IV. In this Section III (including subsections as needed), cover for example and if appropriate cytogenetics (*e.g.* karyology, meiotic behavior), nuclear genome size, possible extent of repetitive or non-coding DNA sequences, main genetic diversity or variability (*e.g.* among or within populations or varieties, and of alleles at a locus), evidence of heterosis or inbreeding depression, maternal and/or paternal inheritance of organellar genomes, and methods of classical breeding (*e.g.* utility from employing mutagenesis with the species). The relevance of the information to the species' variability and the potential effects of transformation are paramount in deciding what to include, as the focus is not to provide this genetic characterisation for plant development.

Intraspecific crossing with both non-cultivated strains (*e.g.* weedy races) and among non-transformed cultivars is appropriately covered here (perhaps with a table or diagram), including any genetic or cytoplasmic constraints or limitations to crossing (*e.g.* cytoplasmic or nuclear sterility, incompatibility systems). Interspecific crosses are addressed in the following section.

> ***Rationale***: The information in this section includes genetic and breeding data, such as details of genomic or genetic stability (including gene silencing) and intraspecific outcrossing behaviour and potential, only to the extent that such information describes parameters that influence how genetic material (including new material) behaves in particular genetic backgrounds, and in outcrossing. Interspecific hybridisation is in a separate section (which follows) because intraspecific crossing is more likely (and familiar), and interspecific hybrids may bring in broader or more extensive concerns.
>
> ***Examples***: OECD SHROB No. 9 (bread wheat, Sections III & V, pp. 13-17 & 20-24); No. 12 (Norway spruce, Section VI, pp. 21-23); No. 13 (white spruce, Section V, pp. 22-24); No. 14 (rice, Section VI, pp. 23-25); No. 24 (*Prunus* spp. – stone fruits, Section II, pp. 15-20); and No. 31 (sunflower, Section IV, pp. 27-28).

4. Hybridisation and introgression

4.1. Natural facility of interspecific crossing (extent, sterility/fertility)

Describe interspecific (including intergeneric) crosses observed under natural conditions. Provide a list and perhaps a diagram of the documented hybrids, *i.e.* the crossings that may occur unaided under usual environmental conditions — if the crossable relatives (other species) might be present. The information could include a discussion of ploidy (and ancestral/progenitor genomes). Provide an indication or review of the likelihood of first-generation (F_1) hybrids and later generations of these F_1 hybrids, and as well whether the F_1 hybrids may be bridges for genes to cross into other (non-parental) species. Rare plant species are considered here and in the following subsection. Indicate naturally hybridising species that are weedy (including invasive) in the list of hybridising species (detailed discussion of their weediness in a local environment would be covered in an environmental risk/safety assessment).

> ***Rationale***: The ability of a cultivated species to hybridise with other cultivated or wild species is a significant factor in determining whether genes or traits could be transferred to other species.
>
> ***Examples***: OECD SHROB No. 7 (oilseed rape, Section VII, pp. 18-21); No. 9 (bread wheat, Section V, pp. 20-24); and No. 16 (poplars, Sections III & VI, pp. 20 & 28-29).

4.2. Experimental crosses

Discuss the experimental data available on outcrossing under controlled conditions, and theoretical possibilities for and barriers to outcrossing. This information is in contrast to that in the previous subsection, which indicates the outcrossing to readily crossable relatives. Experimental data that is the result of forced crosses employing special techniques (*e.g.* embryo rescue) would be relevant only if such studies help to clarify degree of relatedness and likelihood of natural crossing. Theoretical considerations or experimental information might be, for example, on cytogenetic data and meiotic behaviour, or sexual incompatibility systems.

> ***Rationale***: Experimental data and theoretical considerations may broaden the understanding of potential (or as yet unknown) unaided (natural) gene transfer. The information and data are only relevant if unaided crossing in the field can occur.
>
> ***Examples***: OECD SHROB No. 8 (potato, Section VII, pp. 19-21); No. 13 (white spruce, Section VI, pp. 25-26); No. 16 (poplars, Section VI, pp. 28-29); and No. 22 (eastern white pine, Section IV, p. 17).

4.3. Information and data on introgression

Provide an indication or review of the likelihood of F_1 hybrids backcrossing into one or both parents. Provide information on both natural and experimental introgression (extensive backcrossing), and on the (types of) genes or the traits for which introgression has been demonstrated. For example, extensive backcrossing and introgression may be only in one direction, rather than into both parental lines or species' populations. Information should include the extent of likely natural (*i.e.* unaided) introgression or generations of experimental backcrossing, and the fertility and fecundity of the resultant plants.

> ***Rationale***: Of primary consideration is whether interspecific crossing will lead to the introgression of genes. Interspecific crossing is a necessary but typically not a sufficient step for considerable introgression to occur. Even if introgression occurs, it is not the presence but the expression of the gene or trait that may be of primary importance.
>
> ***Examples***: OECD SHROB No. 7 (oilseed rape, Section VII, pp. 20-21); No. 24 (*Prunus* spp. – stone fruits, Section II, p. 30); and No. 31 (sunflower, Section IV, pp. 28-29).

5. General interactions with other organisms (Ecology)

5.1. Interactions in natural ecosystems, and in agronomic, silvicultural or other ecosystems where the species is cultivated or managed

Provide a general overview (including subsections as needed) of main functional ecological interactions of the species of interest within these natural and managed ecosystems and habitats, for example symbiotic relationships, food webs (*e.g.* fruit and seed consumers or predators), noxious/toxic or other important interactions with insects (*e.g.* chemical defense) and other animals, and with plants (*e.g.* allelopathy). Tritrophic interactions may also be considered. Subsections 1.3.2 and 1.3.3 list and briefly characterise the natural (unmanaged) and managed ecosystems and habitats in which the species of interest occurs. The importance of a pollination system to the animal pollinator is detailed here, whereas the importance to the plant is addressed in Subsection 2.2.2. A listing of pertinent pests and pathogens (and diseases) may be presented as an appendix, with only those that are critically relevant discussed here.

> ***Rationale***: The description of the basic general ecology of the species of interest is useful when determining the scope of interactions that may be used as a baseline for understanding the influences the cultivated plant may have on organisms that are in usual close contact.

A general understanding of the interactions of the species with other organisms will aid in determining whether any concerns may arise with a change in the genetics of the species.

Examples: OECD SHROB No. 7 (oilseed rape, Section VII & Appendix, pp. 21 & 29) and No. 13 (white spruce, Section VII, pp. 28-31).

6. Human health and biosafety

6.1. Plant characteristics relevant for human health

Provide brief information on major natural toxicants and common allergenic or medicinal properties of the plant. In some cases, it may be relevant to mention similar information from related species (*e.g.* glycoalkaloids in crossable wild relatives of *Solanum tuberosum* subsp. *tuberosum*, potato).

Rationale: This theme can be regarded as human ecology, a subset of Section V that warrants coverage separately. Baseline information is briefly described, relating to human health as it might be affected by cultivation of the plant (*e.g.* levels of latex or psoralen). Potential effects on human health would be thoroughly treated elsewhere, such as in an OECD plant compositional consensus document for dietary issues.

Example: OECD SHROB No. 8 (potato, Section IV, p. 14).

7. Additional information

The possibility is expressly left open for topics of additional information that is pertinent to environmental risk/safety assessment, as a section in the main text of the document, and/or as appendices.

8. References

As much as possible, the references should be peer-reviewed literature available internationally. After the references directly cited in the text, this section could include a subsection on additional useful references ‘for further reading’.

Example: OECD SHROB No. 7 (oilseed rape, Section IX, pp. 27-28).

Appendix I – Common pests and pathogens

Provide a list of causative organisms for diseases (pathogens) and pests that commonly occur in the crop under agronomic, silvicultural, or equivalent conditions.

Rationale: Provide as considered useful for risk/safety assessment rather than usual production management. Critically important organisms and ecological relationships (*e.g.* a virus disease that is a principal management issue) are covered in Section V. The risk/safety assessment would then consider whether the transformation in the crop would be of environmental concern.

Examples: OECD SHROB No. 18 (sugar beet, Appendix, pp. 32-37 and No. 31 (sunflower, Section V & Appendices 1 & 2, pp. 31 & 37-47).

Appendix II – Biotechnological developments

General information on the kinds of traits being introduced into the species may be included. Provide information directly necessary for defining the scope or detail of biological information that would be useful. For example, transgenes under experimental development for a crop might result in a change in environmental fitness or range and habitats of the plant or its relatives (*e.g.* disease resistance, and

drought, frost or salinity tolerance). Other biotechnological developments (*e.g.* to assist in marketing) may not be pertinent to address here.

Rationale: An overview of biotechnological developments may help to assure that the biological information included in a consensus document is pertinent to the environmental risk/safety assessments anticipated. Consensus documents that include the biotechnological developments to bring traits into the crop can be quite useful in explaining the relevance of assessing certain kinds of biosafety information.

Examples: OECD SHROB No. 14 (rice, Appendix III, pp. 42-45) and No. 27 (maize, Appendix A, pp. 39-41).

Part 1.

Consensus documents on the biology of trees

Section 1.
Western white pine (*Pinus monticola*)

1. Taxonomy

The largest genus in the family Pinaceae, *Pinus* L., which consists of about 110 pine species, occurs naturally through much of the Northern Hemisphere, from the far north to the cooler montane tropics (Peterson, 1980; Richardson, 1998). Two subgenera are usually recognised: hard pines (generally with much resin, wood close-grained, leaf fascicle sheath persistent, two fibrovascular bundles per needle — the diploxylon pines); and soft, or white pines (generally little resin, wood coarse-grained, sheath sheds early, one fibrovascular bundle in a needle — the haploxylon pines). These subgenera are called respectively subgenus *Pinus* and subgenus *Strobus* (Little and Critchfield, 1969; Price *et al*., 1998; Gernandt *et al*., 2005). Occasionally, one to about half the species (20 spp.) in subgenus *Strobus* have been classified instead in a variable subgenus *Ducampopinus*.

Western white pine (*Pinus monticola* Dougl. ex D. Don) belongs to subgenus *Strobus* (Syring *et al.*, 2007). *Pinus monticola* was classified by Critchfield and Little (1966) as one of 14 white pines in section *Strobus*, subsection *Strobi*, now call section *Ouinquefoliae* and subsection *Strobus,* respectively. Earlier classifications have varied in the number of species assigned to subsection *Strobus*, but *P. monticola* has consistently been grouped with the New World species *P. ayacahuite, P. lambertiana,* and *P. strobus* and the Old World species *P. wallichiana* (synonym *P. griffithii*) and *P. peuce* (Critchfield, 1986).

A molecular phylogeny of the genus *Pinus,* based on the nuclear ribosomal DNA internal transcribed spacer (nrITS), did not support separation of subsection *Strobus* from either subsection *Cembrae* or subsection *Krempfianae* (Liston *et al*., 1999). While this study did not include *P. monticola*, it included close relatives such as *P. strobus*. Recent research based on chloroplast DNA sequences recognises an enlarged subsection *Strobus* (*sensu lato*) that absorbs subsection *Cembrae*, while retaining the Asian subsection *Krempfianae* (Gernandt *et al*., 2005). The lack of clear differentiation between subsections *Cembrae* and *Strobus* (*sensu stricto*) is also evident in the ability of *P. monticola* to hybridise with some, but not all species in subsection *Strobus*, and some, but not all species in subsection *Cembrae* (see Section V below). These two subsections had been separated primarily by the nearly wingless seeds and their retention in cones in subsection *Cembrae*, but these bird-dispersal traits appear to have evolved several times rather than once (Critchfield, 1986).

No subspecies or varieties are recognized for western white pine. Nonetheless, populations in the Sierra Nevada, Klamath and Warner mountains in the southern portion of its range have been observed to differ substantially from those farther north (Steinhoff *et al*., 1983).

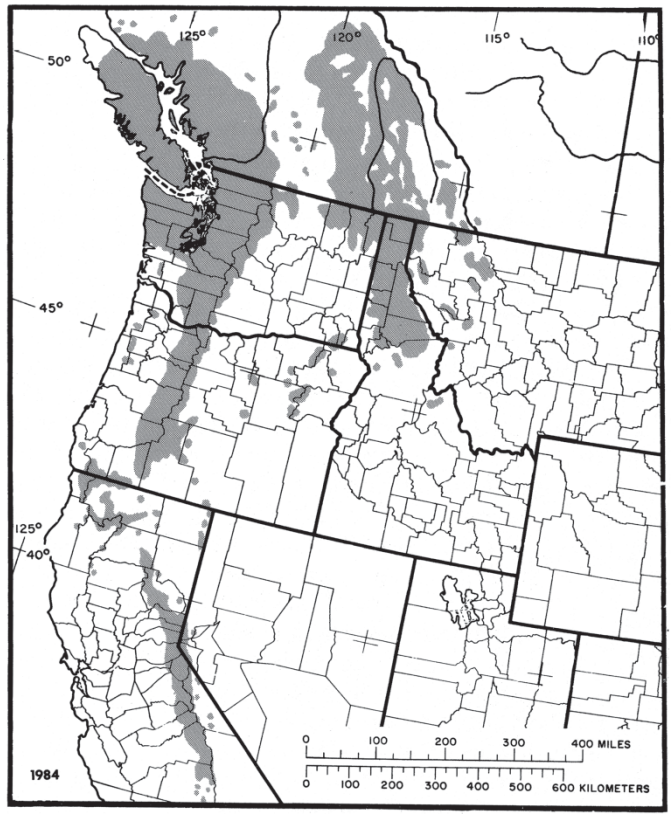
2. Natural distribution

Western white pine is a commonly occurring Western North American species. It is distributed mainly in the central and southern portion of the Cordilleran region and in the central portion of the Pacific region (Klinka *et al*., 2000) (Figure 1). Western white pine grows along the west coast from latitude 36ºN in southern Tulare County, California, USA to 51º30' N near Bute Inlet in southern British Columbia, Canada.

Along the west coast of North America the species grows on Vancouver Island, on the adjacent mainland, southward through Washington and Oregon, and in the Cascade Mountains (Critchfield and Little, 1966). It is also found in the Siskiyou Mountains of southern Oregon and northern California, in the Sierra Nevada of California, and near Lake Tahoe, Nevada.

In the interior, western white pine grows from 52°30' N near Quesnel, British Columbia, southward through the Selkirk Mountains of eastern Washington and northern Idaho, and into the Bitterroot Mountains of western Montana. Its southernmost interior limit is in the Blue Mountains of northeastern Oregon (latitude 44°14' N). Isolated populations are found as far east as Glacier National Park, Montana. It attains its greatest size in the interior portion of the range, which includes northern Idaho and the adjacent sections of Montana, Washington, and British Columbia (Wellner, 1965).

Figure 1. The native range of western white pine



Source: from Graham, 1990

3. Reproductive biology

3.1. Reproductive development

Western white pine is monoecious. Reproductive buds differentiate during July and August; then buds open and strobili appear in June of the following year. Male strobili are about 10 cm long, borne in clusters of 15 to 25 on branches in the middle of the crown. Pollen is shed starting in late June, and can continue until mid-July, but usually averages 8 days (Wellner, 1965). Female strobili are borne on stalks in the upper crown. The erect seed cones are 1.5 cm to 4.0 cm long at the time of pollination, and grow to 2.5 cm to 5.0 cm by the end of the first growing season.

After wind dispersal, the saccate pollen grains initially adhere to lipid microdrops of the micropylar arms of female strobili. A pollination drop is then secreted from the ovule, as in other pines, and it accumulates pollen. The pollination drop can be enhanced through artificial misting. Pollen lands on the drop and is withdrawn into the micropyle (Owens *et al*., 2001). After pollination, germination occurs and pollen tubes penetrate approximately one third of the way through the nucellus. The generative cell and tube nucleus move into the pollen tube, and the megagametophyte initiates free division. The cones and pollen tubes then enter a dormant state around mid-July. Growth does not resume until the following April, when pollen tubes complete growth, the generative cell divides mitotically to produce two sperm nuclei, and fertilisation occurs by the end of May. Each ovule has three to five archegonia (Owens and Bruns, 2000). Multiple fertilisation events can produce multiple proembryos, but mature seeds typically contain only one embryo.

The timing of anthesis can vary up to 3 weeks, and is controlled by temperature during the previous weeks. Anthesis is delayed about 5 days per 300 m increase in elevation, and by about 11 days per degree Celsius below normal temperatures for May and June. In northern Idaho, good cone crops occur every 3 to 4 years (Wellner, 1965). Warm, dry stress periods, during the early summer 2 years before strobili emerge favour the differentiation of reproductive buds. In contrast, stresses in the late summer when reproductive buds are forming or during the period of emergence depresses production of reproductive buds.

3.2. Mating system and gene flow

Western white pine is a predominantly outcrossing species, typical of most conifers. Single and multi-locus estimates of outcrossing over 3 years were all over 0.92 (El-Kassaby *et al*., 1993). Relatively high rates of inbreeding depression, polyembryony and spatial separation of male and female strobili all likely play a role in reducing effective self-pollination, although no phenological barriers to selfing appear to exist (Bingham *et al*., 1972).

The strong differentiation between populations in the Sierra Nevada of California and the mountains of southern Oregon and those in the remainder of the range indicates little gene flow occurs between these regions (Steinhoff *et al.,* 1983). The lack of strong differentiation among central and northern populations might suggest high levels of gene flow, but may just reflect a common origin of these populations from a single Pleistocene refugium (Critchfield, 1984).

3.3. Seed production

Cones of western white pine become mature during August and September of the second year after reproductive buds differentiate. Ripe cones range from yellowish- to reddish-brown (Krugman and Jenkinson, 1974). Mature cones are usually 20 to 25 cm long, although they can vary from 5 to 36 cm (Graham, 1990).

Trees as young as 7 years of age can produce seed cones, and trees become more productive with age. Cone production does not become frequent and abundant until trees are about 70 years. Seed production increases with age and size until trees are about 50 cm in diameter. After that, seed production depends on individual tree vigour, crown shape, and genetics (Wellner, 1965; Owens and Fernando, 2007).

Individual cones can contain more than 300 seeds, but averaged 226 in an 18-year study (Bingham and Rehfeldt, 1970). Western white pine seeds are relatively large for a temperate conifer, averaging 59,000 seeds per kg and ranging from 30,900 to 70,500 seeds per kg (Krugman and Jenkinson, 1974). The seed rain per hectare can be high but variable. In northern Idaho, stand-level seed production varied from 41,000 to 457,000 seeds per ha (Graham, 1990).

Seeds are released by the flexing of cone scales from early fall through winter, with 15% shed before September, 70% shed during September and October, and 15% shed during the late fall and winter. The winged seeds are usually dispersed by wind, but squirrels, mice, and various birds also transport seed. Most seeds fall within 120 m of the seed parent tree, but some have been known to travel over 800 m (Wellner, 1965).

Western white pine seeds can remain viable for a few years in the forest floor. Seeds have shown 40% viability after one winter, 25% viability after two winters, and less than 1% after 3 or 4 years in the forest floor (Graham, 1990). When properly dried and cold stored, western white pine seeds can remain viable for at least 20 years (Krugman and Jenkinson, 1974).

A number of cone and seed insects can cause partial to almost complete failure of cone crops in years with poor or moderate crops. Substantial seed losses result from cone beetles (*Conophthorus monticolae* and *C. labertianae)* and cone moths (*Dioryctria abietivorella* and *Eucosma rescissoriana)* (Furniss and Carolin, 1977). Western white pine seeds are also eaten by both red squirrels (*Tamiasciurus hudsonicus*) and deer mice (*Peromyscus maniculatus*).

3.4. Natural regeneration

Both fresh and stored seed require 30 to 120 days of cold stratification at temperatures of 1º to 5ºC to break dormancy and obtain good germination rates (Krugman and Jenkinson, 1974). Seed dormancy appears to be controlled by the seed coat or nucellus as well as embryo or gametophyte physiology (Hoff, 1986a). Germination is epigeal, as in all pines. There is a strong genetic component to seed germination (Graham, 1990). The nucellus and seed coat may limit water entry or gas exchange (Dumroese, 2000). Clipping of a portion of the seed coat and nucellus can increase germination rate or reduce the stratification requirement (Hoff, 1986a).

The seeds of western white pine usually germinate in the spring when soil is wet from melting snow. Exposed mineral soil is a better seedbed than organic matter even though the forest floor may contain many more stored seeds (Graham, 1990). Germination starts in April at lower elevations, and can continue on exposed sites until July and on protected sites until August (Graham, 1990). Germination begins and ends much earlier in full sunlight than in shade. Soil temperature appears to control the initiation of germination, and dry mineral or organic soil can inhibit germination (Wellner, 1965).

During the first growing season, seedling mortality is high due primarily due to disease, but insects, rodents, and birds can also cause mortality. *Fusarium*, the cause of a damping-off disease, and *Neopeckia coulteri*, a snow mold, are major agents of mortality (Hepting, 1971). *Rhizina undulate* can kill seedlings up to 5 years old. Abiotic stresses cause most seedling mortality late in the first growing season, primarily to temperature and drought. For the most part, western white pine seedlings have low drought tolerance (Minore, 1979). High surface temperatures result in seedling mortality on exposed sites, and drought is sometimes problematic under shady conditions where root penetration is slow, and therefore inadequate to capture adequate soil moisture.

On severe sites, partial shade promotes seedling establishment while on northern aspects and more sheltered sites, full sun is preferable. Due to its relative shade intolerance, western white pine grows best in full light on all sites once established (Wellner, 1965).

Early growth of western white pine seedlings, both above and below ground, is usually moderate. In the first summer, the primary root grows from 5 to 50 cm, depending on light, nutrients and moisture. Growth in height is much less than roots, with seedlings averaging 3 to 5 cm by the end of the first growing season. In northern Idaho, open-grown western white pine seedlings reach a height of about 1.4 m in about 8 years (Graham, 1990).

In northern Idaho, western white pine initiates both height and diameter growth in early May. In British Columbia, shoot elongation usually ends by early August and buds are usually set by mid-August (Schmidt and Lotan, 1980).

Western white pine is usually managed under even-aged silvicultural systems (Burns, 1983). Clearcutting can be followed by natural regeneration, planting of seedlings, or a combination of both. Although success of natural regeneration is high, the advantage of planting is in providing an excellent opportunity for planting of genetically improved, rust-resistant stock and initial stocking control (Fins *et al*., 2001). Successful natural regeneration requires adequate seed source, appropriate seedbed, and suitable microsites.

3.5. Vegetative reproduction

Western white pine does not layer or sprout naturally. Stem cuttings from trees more than 4 to 5 years old root with poor success (Bingham *et al*., 1972). Auxin promotes rooting of stem cuttings, and this effect can be further enhanced with sucrose. Needle fascicles from 2-year-old seedlings have produced roots and some have produced shoots successfully (Graham, 1990) but fascicles lose the ability to root with maturation of seedling donors (Andrews, 1980).

Western white pine grafts relatively easily with scions from trees of all ages collected from all parts of the crown. Early spring grafting before flushing has the highest success rate. The majority of grafts are compatible, although some incompatibility has been reported (Hoff, 1977). Grafting is generally more successfully when conducted under greenhouse conditions than in the field. Interspecific grafting has been accomplished on other five-needle pine rootstocks, such as eastern white pine (*Pinus strobus*), sugar pine (*P. lambertiana*), and blue pine (*P. wallichiana*) (Bingham *et al*., 1972).

Western white pine has been cloned through tissue culture, both from bud slice explants and *via* somatic embryogenesis. Bud explants have resulted in a relatively low multiplication rate due to the production of relatively few shoots per explant (Lapp *et al.*, 1996). Somatic embryogenesis holds more promise. While relatively few lines that are initiated from single embryos become embryogenic, methods have been developed that yielded at least one successful line per family. The multiplication rate for the successful lines will be large (Percy *et al*., 2000).

4. Genetics

4.1. Cytology

Like other members of the genus *Pinus*, the haploid number of chromosomes is 12 in western white pine (Saylor and Smith, 1966). Chloroplasts are inherited predominantly paternally, while mitochondria are primarily inherited maternally, although some biparental inheritance of organelles can occur (White, 1990; Owens and Bruns, 2000). At the time of fertilisation, maternal plastids are excluded from the neocytoplasm but maternal mitochondria remain. Paternal chloroplasts and a small number of paternal mitochondria are released into the egg from the pollen tube with cytoplasm from the tube cell and generative cell. Maternal mitochondria migrate to and aggregate in the perinuclear zone at the time of fertilisation (Bruns and Owens, 2000).

4.2. Genetic variation

4.2.1. Population-level variability

Populations of western white pine from the Sierra Nevada, southern Cascade and Warner Mountains differ from populations farther north in morphology, growth rate and allozyme frequencies. Variation among populations within these groups for molecular markers is typical for conifers, but surprisingly low

for quantitative traits. Genetic distances and G_{st} values among populations for allozymes are relatively small among populations and regions except for those populations in southern Oregon and California (Steinhoff *et al.* 1983). Genetic distances among populations excluding those in southern Oregon and California were all less than 0.025, whereas the genetic distance between southern and northern populations was 0.075. A subsequent principal component analysis of these data supported the lack of genetic differentiation among regions for all but the southern populations (Guries, 1984), as does variation in terpene composition (Zavarin *et al*., 1990). The lack of strong differentiation among more northern populations for genetic markers may support the hypothesis that this species recolonised the northern portion of its current range from a single glacial refugium in southern Oregon during the last glacial period (Critchfield, 1984).

While the relatively low levels of variation among northern populations for selectively near-neutral genetic markers may not be surprising given the glacial history of this species, the lack of differentiation for quantitative, adaptive traits including cold hardiness, growth rate and phenology is unexpected for a widespread conifer. The high levels of within-population variation argue against the lack of among-population variation over most of the range generated by a demographic event, such as a bottleneck, resulting in a lack of genetic variation to allow population differentiation. Numerous studies have found little variation associated with provenance (Rehfeldt, 1979; Steinhoff, 1979a, 1979b; Rehfeldt *et al*., 1984; Campbell and Sugano, 1989, Chuine *et al.*, 2006). Trees originating from environments as different as northern Idaho and Vancouver Island or the Olympic Peninsula show similar growth and survival in reciprocal transplant studies and other genetic tests (Steinhoff *et al*., 1983). Populations from the coastal and interior portions of the range differ only slightly for cold hardiness and growth, and populations within these regions do not differ substantially for these traits (Thomas and Lester, 1992). The late initiation of primary growth in spring, often not until June, and the very rapid predetermined elongation after initiation may alleviate the need for adaptation of populations for traits relating to phenology and cold hardiness to local climatic factors as is typical of conifers such as Douglas-fir (*Pseudotsuga menziesii*) and lodgepole pine (*Pinus contorta*) (Chuine *et al*., 2006).

4.2.2. Variation among individuals within populations

While variation among populations of western white pine is surprisingly low, within-population variation is high for both genetic markers and for quantitative traits. Within-population variation for allozymes revealed an average of 65% polymorphic loci, 1.7 alleles per locus, and expected heterozygosity of 0.18 within populations (Steinhoff *et al*., 1983).

Within-population genetic variation is high for polygenic traits as well. Heritabilities have been estimated for a variety of traits including white pine blister rust resistance and growth rate. Heritability estimates for resistance based on the bark reaction mechanism are relatively low, with individual heritability (h_i^2) estimated as 0.04, and family heritability (h_f^2) as 0.33 (Hoff, 1986b). The resistance mechanism associated with a low number of needle lesions is under stronger genetic control, with h_i^2 estimated as 0.37 in Idaho (Hoff, 1986b) and as 0.77 in British Columbia (Meagher and Hunt, 1996). Individual heritabilities for growth traits for seedlings growing in raised nursery beds in Oregon were also moderate, ranging from 0.31 to 0.48 for height, and 0.44 to 0.46 for diameter (Campbell and Sugano, 1989). Sapling-aged trees in field tests in British Columbia had an individual heritability of 0.36 for height, while 25-year-old trees in Idaho had much lower estimates for height and diameter of 0.11 and 0.14 respectively (Rehfeldt *et al*., 1991; Bower and Yeh, 1988).

4.3. Inbreeding depression and genetic load

Like most conifers, western white pine has a fairly high genetic load. Self-pollination results in an average of 47 seeds per cone, while control-pollinated outcrossing averages 88 seeds per cone (Bingham and Rehfeldt, 1970). Western white pine exhibits relatively strong inbreeding depression

for growth traits. Progeny resulting from self-pollination grow at 60 to 70% of the rate of progeny of unrelated parents (Bingham *et al*., 1972).

5. Hybridization

Pinus monticola has been successfully hybridised experimentally with four species in the subsection *Strobus* (as narrowly circumscribed by Critchfield and Little, 1966): *P. parviflora, P. peuce, P. strobus,* and *P. griffithii*. Hybridisation has not been limited to within subsection *Strobus* (*sensu stricto*). Filled seed have been produced in artificial crosses with three species in the sometimes-recognised subsection *Cembrae*: *Pinus albicaulis, P. cembra* and *P. korainensis* (Bingham *et al*., 1972). Seedlings have been grown from *P. monticola* x *P. cembra* and *P. monticola* x *P. korainensis* crosses, but died before the crosses could be verified (Bingham *et al*., 1972). Hybrids have been verified between *P. monticola* and both *P. flexilis* and *P. strobiformis*, species sometimes classified in subsection *Strobus* and sometimes in subsection *Flexiles* (Critchfield, 1986). Natural hybrids between *P. flexilis* and *P. strobiformis* have been reported where their native ranges overlap (Kral, 1993). Crosses with *P. armandii*, the white pine least susceptible to blister rust and thus a potential source of genes conferring resistance, have failed, as have crosses with *P. parviflora* (Bingham *et al*., 1972). Crosses to *P. aristata* and *P. balfouriana* in section *Parrya*, subsection *Balfourianae*, have yielded little seed and no seedlings (Bingham *et al*., 1972). Crosses with *P. lambertiana* as the female parent yielded no seed, but the reciprocal cross did produce seed. However, the seedlings from the latter crosses did not exhibit phenotypes intermediate to the parental species (Critchfield, 1986), and they are no longer considered to have been interspecific hybrids (Fernando *et al*., 2005).

Pinus monticola x *P. strobus* seedlings are vigorous, and grow much more rapidly than *P. monticola*, although the relative growth advantage is less for sapling-aged hybrids. Hybrids with *P. griffithii*, *P. flexilis* and *P. strobiformis* have also grown well at Placerville, California (Bingham *et al*., 1972).

6. Ecology

6.1. Climate

Western white pine grows in a variety of wetter climates, both maritime and continental, ranging from subalpine boreal (less frequent) to temperate (frequent) to mesothermal (frequent) (Klinka *et al*., 2000). There are three major regions within the species range, differing in climate: 1) Vancouver Island and the Cascade and Siskiyou Mountains; 2) the interior portion of the range, comprising northern Idaho, northern Montana, northeastern Washington and southwestern British Columbia; and 3) the Sierra Nevada of California.

Vancouver Island, the Cascade Mountains, and the Siskiyou Mountains have cool maritime climates, with wet winters and dry summers. Precipitation varies considerably across this region with elevation and the orientation of the mountain ranges. Latitudinal variation from Oregon through British Columbia is relatively small (Shumway, 1979). Precipitation ranges from 1500 to 2010 mm on Vancouver Island and in the Cascade Mountains to 510 to 1520 mm per year in the Siskiyou Mountains. Deep, heavy snowpacks develop over 600 m in elevation. Temperatures range from a low of -18ºC to a maximum of 38ºC (Graham, 1990).

In the parts of the Sierra Nevada where western white pine grows, mean annual precipitation ranges from 760 to 1500 mm, and most of this falls as snow. The temperature averages -9ºC in February and 27ºC in July and August, with maximum temperatures near 37ºC.

Despite being 400 km from the Pacific Ocean, the climate of the interior portion of the range is still under a maritime influence. Annual precipitation is between 710 and 1520 mm, with little of this during

the summer. Snowfall averages 262 cm and ranges from 122 cm to 620 cm. Mean annual temperature in the interior ranges from 4ºC to 10ºC with extremes from -40ºC to 40ºC (Wellner, 1965).

6.2. Soils

Western white pine, a calciphytic species, tolerates a relatively wide range of soil moisture conditions, ranging from moderately dry to wet, and a somewhat narrower range of soil nutrient conditions, ranging from medium to very rich soils. The most productive growth occurs on fresh to moist, nitrogen-rich soils. Compared to other Pacific Northwest tree species, it does not tolerate water- and nutrient-deficient soils, but can tolerate water-surplus and inundated soils (Krajina, 1969; Klinka *et al*., 2000).

Many young western white pine trees suffer mortality in strongly leached, calcium-poor soils in wet climates. When trees are already showing signs of calcium deficiency, their roots are readily killed by summer drought. Plants experimentally subjected to calcium deficiency frequently wilt, even when water is available. In other cases of calcium deficiency, western white pines die more slowly, from the top down, exhibiting chlorosis and later necrosis (Krajina, 1969).

A variety of soils support western white pine along the Pacific Coast. The species grows best on deep, well-aerated soils but is most common on coarse-textured soils. The soils are derived from a variety of parent materials and are generally shallow to moderately deep with medium acidity. Organic matter content is usually low to intermediate, and textures range from sandy loam to clay loam. The majority of the soils in which western white pine grow are Spodosols.

The soils on which western pine grows in the interior portion of the range are also diverse and predominantly Spodosols that have developed from weathered granite, schist, quartzite, argillite, sandstone, and shale. Soil depths range from 25 to over 230 cm. The upper soil layer is often composed of loess or loess-like material (Cooper *et al*., 1987). In British Columbia, soils have developed from base-rich glacial materials (till, fluvial, or lacustrine deposits) (Wellner, 1965).

Western white pine grows from sea level to subalpine elevations, and on a variety of slopes and aspects. It is most common on lower slopes, along creeks, northerly aspects and alluvial terraces (Graham, 1990).

6.3. Synecology

Depending on site and disturbance history, western white pine grows predominantly as a minor (infrequently as a major) species in even-aged, mixed-species stands, and is present in all stages of secondary succession. Occasionally, it is a minor component in transition old-growth stands on calcium-rich soils in cool temperate and cool mesothermal climates. As a moderately shade-tolerant species, it is considered a persistent seral species which attains a dominant position in the stand only following wildfires, using even-aged silviculture systems, or through stand treatments favouring the species (Graham, 1990).

Associates of western white pine include *Abies amabilis* (amabilis fir), *A. concolor* (white fir), *A. grandis* (grand fir), *A. lasiocarpa* (subalpine fir), *A. magnifica* (red fir), *A. procera* (noble fir), *Acer macrophyllum* (bigleaf maple), *Alnus rubra* (red alder), *Arbutus menziesii* (Pacific madrone), *Betula papyrifera* (white birch), *Chamaecyparis lawsoniana* (yellow-cedar), *Larix occidentalis* (western larch), *Libocedrus decurrens* (incense cedar), *Picea engelmannii* (Engelmann spruce), *P. sitchensis* (Sitka spruce), *Pinus balfouriana* (foxtail pine), *P. contorta* (lodgepole pine), *P. flexilis* (limber pine), *P. jeffreyi* (Jeffrey pine), *P. lambertiana* (sugar pine), *P. ponderosa* (ponderosa pine), *Pseudotsuga menziesii* (Douglas-fir), *Thuja plicata* (western redcedar), *Tsuga heterophylla* (western hemlock), and *T. mertensiana* (mountain hemlock) (Franklin and Dyrness, 1973; Eyre, 1980; Graham, 1990; Klinka *et al*., 2000).

Western white pine is found in eighteen of the forest cover types of western North America (Eyre, 1980). It is the dominant species in the Western White Pine cover type (Type 215). The western white pine component in this type is usually even-aged with an understory containing multi-aged trees of the more shade-tolerant softwoods; occasionally, a minor component of other shade-intolerant softwoods may also be present in the upper canopy. Western white pine is a common but minor component, along with many other tree species, in seventeen other cover types: Mountain Hemlock (205), Engelmann Spruce–Subalpine Fir (206), Red Fir (207), Interior Douglas-Fir (210), Western Larch (212), Grand Fir (213), Lodgepole Pine (218), Western Hemlock (224), Coastal True Fir–Hemlock (226), Western Red Cedar–Western Hemlock (227), Western Red Cedar (228), Pacific Douglas-Fir (229), Douglas-Fir–Western Hemlock (230), Port-Orford-Cedar (231); Interior Ponderosa Pine (237); Jeffrey Pine (247), and California Mixed Subalpine (256) (Eyre, 1980).

The cover and composition of understory vegetation in all these cover types will vary depending on site (climate and soil), associated tree species, stand developmental stage, and stand density. Relative to other tree species, light interception by western white pine is low, thus providing favourable light conditions for the development of diverse understory vegetation.

6.4. Stand dynamics

Western white pine is dependent on periodic wildfires. Rapid growth and longevity have enabled western white pine to persist as a widespread element in Pacific Northwest forests. Without major disturbances such as fire or timber harvesting, western white pine would be replaced over time by more shade-tolerant conifers (Franklin and Dyrness, 1973; Graham, 1990). Trees are generally long-lived, with many individuals living 300 to 400 years, and rarely up to 500 years. Old trees are often more than 180 cm in diameter and 60 m tall (Graham, 1990; Klinka *et al*., 2000).

Western white pine can be naturally regenerated using even-aged silviculture such as seed tree or shelterwood systems. Adequate natural regeneration usually develops within 5 to 10 years of harvest. Without a naturally blister rust-resistant seed source on a site, planting should be used to regenerate the stand after harvest. In shelterwoods, growth will be markedly reduced if the overstory is dense and its removal is delayed (Wellner, 1965).

The composition of mixed stands containing western white pine is determined during the first 30 years after regeneration (Graham, 1988; Jain *et al*., 2004). Young lodgepole pine and western larch can grow considerably faster in height than juvenile western white pine. Lodgepole pine's growth superiority usually disappears by age 50, but western larch can usually maintain a height advantage over western white pine. Grand fir can match western white pine's height growth for the first 30 years, and Douglas-fir has similar height growth. On northerly aspects and in shaded conditions, shade-tolerant western hemlock can also equal the height growth of western white pine (Deitschman and Pfister, 1973).

6.5. Damaging agents

Western white pine has relatively thin bark, moderately flammable foliage and highly flammable cones, making it intermediate in fire resistance among its coniferous associates (Minore, 1979), yet it depends on fire or logging to remove competing conifers. As a result of both fire protection and blister rust infection, the proportion of western white pine regeneration (planted and natural) in northern Idaho, eastern Washington, and western Montana decreased from 44% in 1941 to 5% in 1979 (Graham, 1990). Between 1976 and 1996, approximately 100,000 ha in the Inland Northwest were replanted with blister rust resistant stock from the Idaho breeding programme (Fins *et al*., 2001).

Dormant western white pine is, along with lodgepole pine, one of the more cold-hardy western North American conifers. Needle desiccation can result from winds or sun causing excessive moisture loss during times when soil is frozen or cold. Western white pine is quite tolerant of heat compared to

most of its shade-tolerant associates. It is relatively wind-firm but snow breakage is common in pole-stage stands (Graham, 1990).

The most serious of the diseases infecting western white pine is white pine blister rust, caused by the pathogen *Cronartium ribicola* (Hepting, 1971). In northern Idaho and adjacent regions, a favourable climate and abundant *Ribes* alternate hosts contribute to heavy losses. However, selection and breeding of naturally rust-resistant parent trees for the planting of rust-resistant nursery stock has been successful. Other stem diseases are of little consequence.

A physiological disorder called pole blight can result from extended periods of drought (Graham, 1990). Symptoms include yellow foliage, necrotic resinous areas on the trunk, and top or whole tree death. This disease appears to be caused by root deterioration in soils restricting water uptake (Leaphart, 1958; Leaphart and Stage, 1971)

The principal root disease of western white pine is caused by *Armillaria* spp., resulting in dieback of foliage, reductions in growth, resin exudates at the root collar, and black rhizomorphs. *Heterobasidion annosum* and *Phellinus weirii* also cause some mortality. *Phellinus pini, Heterobasidion annosum,* and *Phaeolus schweinitzii* are the most damaging butt rot fungi (Hepting, 1971).

The bark beetle *Dendroctonus ponderosae* (mountain pine beetle) is the most significant insect pest of western white pine. Bark beetles kill groups of mostly mature trees weakened by blister rust (Furniss and Carolin, 1977).

7. Forestry practices

7.1. Deployment of reforestation materials

Western white pine is grown within most of its range using even-aged silvicultural systems. Clearcut, seed-tree, and shelterwood cuts result in adequate and diverse natural regeneration within 5 to 10 years of harvesting (Burns, 1983; Graham, 1990). If a natural white pine blister rust resistant seed source is not present on the site, planting must be used to regenerate the species. When natural regeneration of clearcuts is used for establishing mixed species stands which include western white pine, it is common to regenerate 11,000 trees per hectare, 1,000 of which are western white pine. Similarly, seed-tree cuts can produce 12,000 trees per hectare, 1,500 of which are western white pine. Shelterwood harvesting produces more trees, but the proportion of western white pine is less than for other silvicultural systems that produce higher light levels for regeneration (Boyd, 1969). Western white pine is not sufficiently shade tolerant for individual-tree selection cuts. Group selection cuts may have limited application.

The introduced pathogen *Cronartium ribicola*, which causes white pine blister rust, has driven reforestation decisions for western white pine. Propagation and planting of resistant seedlings is the primary method for regenerating western white pine. Techniques for collection, processing, testing, and storage of seed are given in Krugman and Jenkinson (1974). The planting of either bare-root or container-grown seedlings on appropriate sites can result in excellent survival and growth. Bare-root stock appears to have better survival when planted in spring than in fall, but containerized seedlings have high survival when planted during either season (Graham, 1990).

The vast majority of seed used for reforestation of western white pine comes from seed orchards containing grafted ramets from white pine blister rust resistant ortets identified in breeding programs in Oregon, Idaho, and to a lesser extent, British Columbia. The frequency of genotypes that are resistant to this disease is very low in the wild, thus the success of plantations originating from wild seed lots is low (Fins *et al*., 2001).

Western white pine was introduced to Europe after 1825, where it was planted in arboretums and parks. In 1880, it was included into a network of experimental plantations by the German Forest Research Institute, but its use as a timber crop species in western and central Europe is very limited (Hermann, 1987).

7.2. Provenance transfer

Western white pine is unusual for a widespread conifer in that it shows little evidence for local adaptation of populations in seedling genecological studies or field provenance trials (Rehfeldt, 1979; Steinhoff, 1979a; Rehfeldt *et al*., 1984; Campbell and Sugano, 1989; Thomas and Lester, 1992). While populations from the Sierra Nevada, California, and the Klamath and Warner mountains in southern Oregon clearly differ from populations farther north, there is little variation among the northern populations (Steinhoff *et al*., 1983). This has permitted large provenance transfers both geographically and elevationally when deploying genetically selected blister rust resistant seed. Although separate seed orchards were initially established for low, mid and high-elevation areas in Idaho, there is little evidence to support management of more than one seed zone (Rehfeldt *et al*., 1984; Mahalovich and Eramian, 1995). Campbell and Sugano (1989) recommended a total of five seed zones for Washington and Oregon.

In British Columbia, there are two seed zones, one for the coastal portion of the range, and one for the interior (Hunt, 1994). Seed imported from seed orchards in Idaho is routinely used for reforestation in southern British Columbia up to 52°N latitude and 1450 m elevation. Seed from wild stand collections in British Columbia in the coastal portion of the range have no provenance transfer limits. Collections in the interior of the province can be transferred a maximum from the collection site to the planting site of 2° latitude to the north, 1° latitude south, 3° longitude east or 2° west, and 700 m up or down in elevation (British Columbia Ministry of Forests, 1995).

7.3. Breeding programmes

The oldest continual breeding program for western white pine was initiated in 1950 in eastern Washington State and Idaho. This program was established as a result of the failed efforts to manage white pine blister rust through the eradication of native *Ribes* spp., the alternate hosts of the disease, and the observation that a small percentage of trees were able to survive in severely infected stands. Phenotypically resistant parents were crossed, and the progeny tested for rust resistance. Resistant seedlings were used to establish a breeding orchard at Moscow, Idaho. These selected trees were then crossed to create the F2 generation for testing and selection. The original breeding orchard was then converted to a seed orchard for seed production for reforestation (Fins *et al.,* 2001). A similar program, modelled after the successful Idaho approach, was initiated at Dorena, Oregon, in 1956 (Sniezko, 1996). A very early breeding program was initiated in British Columbia in the late 1940's, but was abandoned from 1960 until 1984, when a joint provincial-federal breeding program was established (Meagher *et al*., 1990; Hunt, 1994).

Breeding programs typically screen for resistance to *Cronartium ribicola* through artificial inoculation of seedlings with telia of the rust on *Ribes* spp. leaves. Infected leaves are either collected in *Ribes* gardens maintained and inoculated for this purpose, or from plants in the wild. Two-year-old seedlings are placed in a chamber with high humidity and temperatures of 12-18°C. *Ribes* leaves are placed on screens above the seedlings, and sporefall is monitored. When spore fall reaches a threshold level after a day or two, usually 6,000 per cm^{2}, *Ribes* leaves are removed and the seedlings remain in the chamber for an additional 36 hours to allow for spores to germinate (Mahalovich and Eramian, 1995). Seedlings are then placed outside and monitored for rust resistance over a three to five-year period, depending on the program (Hunt, 1990; Sniezko, 1996). Information is also derived from infection and

mortality levels in field genetic tests (Fins *et al*., 2001). Assessments of growth rate are conducted following screening for blister rust resistance (Mahalovich and Eramian, 1995).

There have been many, varied descriptions of rust resistant phenotypes (Hunt, 1997). Early selections in the Idaho program are thought to have been resistant due to a few single-gene (vertical) mechanisms. The emphasis in the program is now to select first for combinations of polygenic (horizontal) mechanisms of resistance, and second for vertical resistance. The Idaho programme has identified eight types of rust resistance in western white pine. Four of these are thought to be controlled polygenically, conferring horizontal resistance: 1) low frequency of needle lesions; 2) early exhibition of stem symptoms; 3) cankers that remain alive over a 3-year period following inoculation; and 4) a high proportion of bark reaction in cankered seedlings 3 years after inoculation. The four remaining types of resistance are thought to be vertical, controlled by single genes: 1) apparent immunity, with no needle lesions following inoculation; 2) abscission of needles with lesions during the first summer after infection; 3) retention of infected needles without the development of a canker; and 4) bark reaction resulting in the termination of canker growth following inoculation. The Idaho program is focussing on selecting families with more than one type of vertical resistance, and selecting individuals within those families exhibiting horizontal resistance (Mahalovich and Eramian, 1995; Fins *et al*., 2001). Families with particular combinations of resistance mechanisms will be grouped into breeding sublines to manage coancestry. The types of resistance recognized in the Oregon and British Columbia breeding programs are similar to the Idaho programme (Meagher *et al*., 1990; Sniezko, 1996). The Oregon programme also plans to combine mechanisms of resistance into breeding lines.

Field genetic tests of F2 improved material in Idaho have mortality rates that average 42% lower than controls (unselected seedlots) over sites with a wide range in blister rust severity. Operational trials of F2 *versus* unimproved stock have yielded similar results, with mortality rates of 7% for improved material and 42% for unimproved stock. Tests have also shown that infection levels vary greatly from one site to another (Fins *et al*., 2001). In coastal British Columbia, progeny of phenotypically selected and tested trees had infection levels of 13% in field trials, while unselected trees had infection levels of 95% and above (Hunt and Meagher, 1989).

The degree of resistance of genetically selected stock varies with site and with races and virulence of *Cronartium ribicola* (Goddard *et al*., 1985; Hoff and McDonald, 1993). The instability of single-gene resistance has been shown by Kinloch and others (1999). They established the single-gene basis of a resistant phenotype with a hypersensitive bark reaction. This form of resistance has already broken down in both *Pinus monticola* and *P. lambertiana* to a virulent race of blister rust in some limited geographic areas in California and Oregon. Idaho F2 seedlings suffered relatively high levels of infection on some sites in coastal British Columbia (Hunt and Meagher, 1989).

Biochemical and morphological differences between white pine blister rust resistant and susceptible phenotypes have been investigated. Bark protein differences have been documented between slow canker growth resistant and susceptible phenotypes (Davidson and Ekramoddoullah, 1997). A protein associated with cold hardiness in western white pine (Pin mIII) has been found to be up-regulated by blister rust infection, possibly reflecting a stress response (Davidson and Ekramoddoullah, 1997; Yu *et al*., 1997; Ekramoddoullah *et al*., 1998). Genotypes with the reduced needle lesion frequency form of resistance appear to have smaller, less round stomata than susceptible genotypes (Woo *et al*., 2001).

The primary objective for breeding programs has been disease resistance, and comparatively little attention has been paid to other traits of interest such as growth rate and wood properties. However, considerable gains for increased growth rate are possible with this species (Rehfeldt *et al*., 1991). As programs advance and high levels of resistance are achieved, more emphasis will be placed on increasing growth rate as a secondary objective.

7.4. Conservation of genetic resources

The level of mortality of young, naturally regenerated trees from wild populations are so high that unlike most tree species in western North America, western white pine genetic resources will not be well-protected *in situ* (Hunt *et al*., 1985; Mahalovich and Eramian, 1995; Fins *et al*., 2001). Genetic conservation in this species will best be protected through a combination of the maintenance of breeding orchards, seed orchards, clone banks and seed banks, and through the aggressive planting of genetically improved, resistant genotypes throughout the natural range of this species. The three breeding programs dedicated to this species all provide such *ex situ* protection of genetic diversity in this species. Slight losses of genetic diversity in this species may occur through breeding and deployment. However, any reductions in overall diversity are likely to be small, and much lower than if genetic conservation relies on wild populations slowly evolve higher levels of resistance, suffering large reductions in numbers of trees in the process and likely leading to the extinction of some populations.

In situ reserves will provide some secondary protection of genetic diversity in western white pine. In British Columbia, a gap analysis of degree of protection of conifers found that this species is fairly well represented in existing parks and ecological reserves, but that outlying populations in a few regions deserved further attention (Lester and Yanchuk, 1996).

8. Summary

Although western white pine is a valuable timber species, it is, and will probably remain, only a minor forest component in western North America. The major hazard limiting its wider application is white pine blister rust. Western white pine is, however, a very productive and desirable species considering its rapid growth, clean bole with minimum taper, narrow crown, and non-resinous wood. Across its range, western white pine functions as a long-lived seral species. It is typically a minor component in the upper canopy of mixed-species, softwood dominated stands at all seral stages. Compared to other pines, it does not tolerate water- and nutrient-deficient sites. Western white pine grows in some of the finest western outdoor recreation areas and has considerable aesthetic value.

Long-term, aggressive breeding programmes for western white pine have achieved substantial gains in resistance to white pine blister rust. These programmes will continue to play a key role in the management of this species. Breeding programmes will need to continue to select for a variety of types of disease resistance, and to emphasize those mechanisms under polygenic control. The breeding programmes also have a major responsibility for genetic conservation as wild populations in protected areas with a high incidence of blister rust may not maintain high enough population sizes for maintenance of genetic diversity or even population persistence. The lack of strong population differentiation or local adaptation, unusual in a widespread conifer, has facilitated the deployment of genetically improved, blister rust resistant seed. Resistant western white pine can be widely deployed to resume a variety of economic and ecological roles in forests in western North America.

References

Andrews, D.S. 1980. Rooting western white pine (Pinus monticola Dougl.) needle fascicles and branch cuttings (vegetative propagation). USDA Forest Service Intermountain Forest and Range Experiment Station Research Note INT-291. Ogden, UT. 11 pp.

Bingham, R.T., and G.E. Rehfeldt. 1970. Cone and seed yields in young western white pines. USDA Forest Service Intermountain Forest and Range Experiment Station Research Paper INT-79. Ogden, UT. 12 pp.

Bingham, R.T., R.J. Hoff and R.J. Steinhoff. 1972. Genetics of western white pine. USDA Forest Service Research Paper WO-12. Washington, DC. 18 pp.

Bower, R.C., and F.C. Yeh. 1988. Heritability and gain calculations for six-year height of coastal western white pine in British Columbia. Can. J. Plant Sci. 68: 1191-1196.

Boyd, R.J. 1969. Some case histories of natural regeneration in the western white pine type. USDA Forest Service Intermountain Forest and Range Experiment Station Research Paper INT-63. Ogden, UT. 24 pp.

British Columbia Ministry of Forests. 1995. Seed and vegetative material guidebook. Forest Practices Code, British Columbia Ministry of Forests, Victoria, BC. 77 pp.

Bruns, D., and J.N. Owens. 2000. Western white pine (*Pinus monticola* Dougl.) reproduction: II. Fertilization and cytoplasmic inheritance. Sex. Plant Reprod. 13: 75-84.

Burns, R.M. (tech. compiler). 1983. Silvicultural systems for the major forest types of the United States. USDA Agric. Handbook 445. Washington, DC. 191 pp.

Campbell, R.K., and A.I. Sugano. 1989. Seed zones and breeding zones for white pine in the Cascade Range of Washington and Oregon. USDA Forest Service Pacific Northwest Research Station Research Paper PNW-407. Portland, OR. 20 pp.

Chuine, I., G.E. Rehfeldt and S.N. Aitken. 2006. Height growth determinants and adaptation to temperature in pines: A case study of Pinus contorta and Pinus monticola. Can. J. For. Res. 36: 1059-1066.

Cooper, S.V., K.E. Neiman, R. Steele and W. David. 1987. Forest habitat types of northern Idaho: A second approximation. USDA Forest Service Intermountain Forest and Range Experiment Station Research Paper INT-236. Ogden, UT. 135 pp.

Critchfield, W.B. 1984. Impact of the Pleistocene on the genetic structure of North American conifers. Pp. 70-118 in Proceedings of the 8th North American Forest Biology Workshop. Dept. of Forest Resources, Utah State University. Logan, UT.

Critchfield, W.B. 1986. Hybridization and classification of the white pines (*Pinus* section *Strobus*). Taxon 35: 647-656.

Critchfield, W.B., and E.L. Little Jr. 1966. Geographic distribution of the pines of the world. USDA Misc. Pub. 991. Washington, DC. 97 pp.

Davidson, J., and A.K.M. Ekramoddoullah. 1997. Analysis of bark proteins in blister rust-resistant and susceptible western white pine (*Pinus monticola*). Tree Physiol. 17: 663-669.

Deitschman, G.H., and R.D. Pfister. 1973. Growth of released and unreleased young stands in the western white pine type. USDA Forest Service Intermountain Forest and Range Experiment Station Research Paper INT-132. Ogden, UT. 14 pp.

Dumroese, R.K. 2000. Germination-enhancing techniques for *Pinus monticola* seeds and speculation on seed dormancy. Seed Sci. Technol. 28: 201-209.

Ekramoddoullah, A.K.M., J.J. Davidson and D.W. Taylor. 1998. A protein associated with frost hardiness of western white pine is up-regulated by infection in the white pine blister rust pathosystem. Can. J. For. Res. 28: 412-417.

El-Kassaby, Y.A., M.D. Meagher and R. Davidson. 1993. Temporal variation in the outcrossing rate in a natural stand of western white pine. Silvae Genet. 42: 131-135.

Eyre, F.H., ed. 1980. Forest cover types of the United States and Canada. Soc. Am. For., Washington, DC. 148 pp.

Fernando, D.D., S.M. Long and R.A. Sniezko. 2005. Sexual reproduction and crossing barriers in white pines: The case between *Pinus lambertiana* (sugar pine) and *P. monticola* (western white pine). Tree Genetics & Genomes 1: 143-150.

Fins, L., J. Byler, D. Ferguson, A. Harvey, M.F. Mahalovich, G. McDonald, D. Miller, J. Schwandt and A. Zack. 2001. Return of the giants. Idaho Forest, Wildlife and Range Experiment Station, University of Idaho. Moscow, ID. 20 pp.

Franklin, J.F., and C.T. Dyrness. 1973. Natural vegetation of Oregon and Washington. USDA Forest Service Pacific Northwest Region General Technical Report PNW-8. Portland, OR. 417 pp.

Furniss, R.L., and V.M. Carolin. 1977. Western forest insects. USDA Misc. Pub. 1339. Washington, DC. 654 pp.

Gernandt, D.S., G. Geada López, S. Ortiz García and A. Liston. 2005. Phylogeny and classification of *Pinus*. Taxon 54: 29-42.

Goddard, R.E., G.I. McDonald and R.J. Steinhoff. 1985. Measurement of field resistance, rust hazard, and deployment of blister rust-resistant western white pine. USDA Forest Service Intermountain Forest and Range Experiment Station Research Paper INT-358. Ogden, UT. 8 pp.

Graham, R.T. 1988. Influence of stand density on western white pine, western redcedar, western hemlock, and grand fir tree and stand development in the Mountain West. Pp. 175-184 in S. Schmidt, ed., Future forests of the Mountain West: A stand culture symposium. USDA Forest Service Intermountain Forest and Range Experiment Station General Technical Report INT-243. Ogden, UT.

Graham R.T. 1990. Pinus monticola. Pp. 385-394 in R.M. Burns and B.H. Honkala (tech. coordinators), Silvics of North America, Vol. 1. USDA Agric. Handbook 654. Washington, DC.

Guries, R.P. 1984. Genetic variation and population differentiation in forest trees. Pp. 119-131 in Proceedings of the 8th North American Forest Biology Workshop. Dept. Forest Resources, Utah State University. Logan, UT.

Hepting, G.H. 1971. Diseases of forest and shade trees of the United States. USDA Agric. Handbook 386. Washington, DC. 658 pp.

Hermann, R.K. 1987. North American tree species in Europe. J. For. 85: 27-32.

Hoff, R J. 1977. Delayed graft incompatibility in western white pine (*Pinus monticola*). USDA Forest Service Intermountain Forest and Range Experiment Station. Ogden, UT. 4 pp.

Hoff, R.J. 1986a. Effect of stratification time and seed treatment on germination of western white pine seed. Pp. 112-116 in R.C. Shearer (compiler), Conifer tree seed in the Inland Mountain West symposium proceedings. USDA Forest Service, Intermountain Forest and Range Exper. Station Research Paper INT-203. Ogden, UT.

Hoff, R.J. 1986b. Inheritance of the bark reaction resistance mechanism in *Pinus monticola* infected by *Cronartium ribicola*. USDA Forest Service Intermountain Forest and Range Experiment Station Research Note INT-361. Ogden, UT. 8 pp.

Hoff, R.J. and G.I. McDonald. 1993. Variation of virulence of white pine blister rust. Eur. J. For. Pathol. 23: 103-109.

Hunt, R.S. 1990. Blister rust in inoculated and plantation-tested western white pine in British Columbia. Can. J. Plant Pathol. 12: 279-282.

Hunt, R.S. 1994. Transferability of western white pine within and to British Columbia – blister rust resistance. Can. J. Plant Pathol. 16: 273-278.

Hunt, R.S. 1997. Relative value of slow-canker growth and bark reactions as resistance responses to white pine blister rust. Can. J. Plant Pathol. 19: 352-357.

Hunt, R.S., and M.D. Meagher. 1989. Incidence of blister rust on resistant white pine (*Pinus monticola* and *Pinus strobus*) in coastal British Columbia plantations. Can. J. Plant Pathol. 11: 419-423.

Hunt, R.S., E. von Rudloff, M.S. Lapp and J.F. Manville. 1985. White pine blister rust in British Columbia. 3. Effects on the gene pool of western white pine. For. Chron. 61: 484-488.

Jain, T.B., R.T. Graham and P. Morgan. 2004. Western white pine growth relative to forest openings. Can. J. For. Res. 34: 2187-2198.

Kinloch, B.B., R.A. Sniezko, G.D. Barnes and T.E. Greathouse. 1999. A major gene for resistance to white pine blister rust in western white pine from the Western Cascade Range. Phytopathology 89: 861-867.

Klinka, K., J. Worrall, L. Skoda and P. Varga. 2000. The distribution and synopsis of ecological and silvical characteristics of tree species of British Columbia's forests. Can. Cartographics Ltd. Coquitlam, BC. 180 pp.

Krajina, V.J. 1969. Ecology of forest trees in British Columbia. Ecol. West. North Am. 2: 1-146.

Kral, R. 1993. *Pinus Linnaeus*. Pp. 373-398 in Flora of North America Editorial Committee, ed., Flora of North America north of Mexico, Vol. 2: Pteridophytes and gymnosperms. Oxford University Press, NY.

Krugman, S.L., and J.L. Jenkinson. 1974. Pp. 598-638 in C.S. Schopmeyer (tech. coordinator), Seeds of woody plants in the United States. USDA Agric. Handbook 450. Washington, DC.

Lapp, M.S., J. Malinek and M. Coffey. 1996. Microculture of western white pine (*Pinus monticola*) by induction of shoots on bud explants from 1- to 7-year-old trees. Tree Physiol. 16: 447-451.

Leaphart, C.D. 1958. Root characteristics of western white pine and associated tree species in a stand affected with pole blight of white pine. USDA Forest Service Intermountain Forest and Range Experiment Station Research Paper INT-52. Ogden, UT. 10 pp.

Leaphart, C.D., and A.R. Stage. 1971. Climate: A factor in the origin of the pole blight disease of *Pinus monticola* Dougl. Ecology 52: 229-239.

Lester, D.T., and A.D. Yanchuk. 1996. A survey of the protected status of conifers in British Columbia: *In situ* gene conservation. British Columbia Ministry of Forests Research Report 04. Victoria, BC. 34 pp.

Liston, A., W.A. Robinson, D. Pinero and E.R. Alvarez-Buylla. 1999. Phylogenetics of *Pinus* (Pinaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences. Mol. Phylogenet. Evol. 11: 95-109.

Little Jr., E.L., and W.B. Critchfield. 1969. Subdivisions of the genus *Pinus* (pines). USDA Forest Service Miscellaneous Publication 1144. 51 pp.

Mahalovich, M.F., and A. Eramian. 1995. Breeding and seed orchard plans for the development of blister rust resistant white pine for the Northern Rockies. USDA Forest Service Northern Region and Inland Empire Tree Improvement Cooperative. 60 pp.

Meagher, M.D., and R.S. Hunt. 1996. Heritability and gain of reduced spotting vs. blister rust on western white pine in British Columbia, Canada. Silvae Genet. 45: 75-81.

Meagher, M.D., R.S. Hunt, E.E. White and A.K.M. Ekramoddoullah. 1990. Improvement of *Pinus monticola* for British Columbia, Canada. Pp. 50-55 in Proceedings of a symposium on white pine provenances and breeding. USDA Forest Service Northeast Forest Experiment Station General Technical Report NE-155. Radnor, PA.

Minore, D. 1979. Comparative autecological characteristics of northwestern tree species – a literature review. USDA Forest Service Pacific Northwest Region Forest and Range Experiment Station General Technical Report PNW-87. Portland, OR. 72 pp.

Owens, J.N., and D. Bruns. 2000. Western white pine (*Pinus monticola* Dougl.) reproduction: I. Gametophyte development. Sex. Plant Reprod. 13: 61-74.

Owens, J.N., and D.D. Fernando. 2007. Pollination and seed production in western white pine. Can. J. For. Res. 37: 260-275.

Owens, J.N., G. Catalano and J.S. Bennett. 2001. The pollination mechanism of western white pine. Can. J. For. Res. 31: 1731-1741.

Percy, R.E., K. Klimaszewska and D.R. Cyr. 2000. Evaluation of somatic embryogenesis for clonal propagation of western white pine. Can. J. For. Res. 30: 1867-1876.

Peterson, R. 1980. The pine tree book. Brandywine Press, NY. 138 pp. + glossary.

Price, R.A., A. Liston and S.H. Strauss. 1998. Phylogeny and systematics of Pinus. Pp. 49-68 in D.M. Richardson, ed., Ecology and biogeography of Pinus. Cambridge University Press, NY.

Rehfeldt, G.E. 1979. Ecotypic differentiation in populations of *Pinus monticola* in north Idaho – myth or reality. Am. Nat. 114: 627-636.

Rehfeldt, G.E., R.J. Hoff and R.J. Steinhoff. 1984. Geographic patterns of genetic variation in *Pinus monticola*. Bot. Gaz. 145: 229-239.

Rehfeldt, G.E., W.R. Wykoff, R.J. Hoff and R.J. Steinhoff. 1991. Genetic gains in growth and simulated yield of *Pinus monticola*. For. Sci. 37: 326-342.

Richardson, D.M., ed. 1998. Ecology and biogeography of *Pinus*. Cambridge University Press, NY. 527 pp.

Saylor, L.C., and B.W. Smith. 1966. Meiotic irregularity in species and interspecific hybrids of *Pinus*. Am. J. Bot. 53: 453-468.

Schmidt, W.C., and J.E. Lotan. 1980. Phenology of common forest flora of the northern Rockies – 1928 to 1937. USDA Forest Service Intermountain Forest and Range Experiment Station Research Paper INT-259. Ogden, UT. 20 pp.

Shumway, S.E. 1979. Climate. Pp. 87-91 in P.E. Heilman, H.W. Anderson and D.M. Baumgartner, eds., Forest soils of the Douglas-fir region. Cooperative Extension Service, Washington State University. Pullman, WA.

Sniezko, R.A. 1996. Developing resistance to white pine blister rust in sugar pine in Oregon. Pp. 171-178 in B.B. Kinloch, M. Marosy and M.E. Huddleston, eds., Sugar pine: Status, values and roles in ecosystems. Proceedings of a symposium. University of California, Division of Agric. and Nat. Res., Davis, CA. Pub. 3362.

Steinhoff, R.J. 1979a. Variation in early height growth of western white pine *Pinus monticola* in North Idaho. USDA Forest Service Forest Intermountain Service Forest and Range Experiment Station Research Paper INT-222. Ogden, UT. 22 pp.

Steinhoff, R.J. 1979b. Variation in western white pine *Pinus monticola*. Pp. 52-54 in Proceedings of the annual Western Interior Forest Diseases Working Conference. USDA Forest Service Rocky Mountain Forest and Range Experiment Station. Fort Collins, CO.

Steinhoff, R.J., D.G. Joyce and L. Fins. 1983. Isozyme variation in *Pinus monticola*. Can. J. For. Res. 13: 1122-1132.

Syring, J., K. Farrell, R. Businský, R. Cronn and A. Liston. 2007. Widespread genealogical nonmonophyly in species of *Pinus* subgenus *strobus*. Syst. Biol. 56: 163-181.

Thomas, B.R., and D.T. Lester. 1992. An examination of regional, provenance, and family variation in cold hardiness of *Pinus monticola*. Can. J. For. Res. 22: 1917-1921.

Wellner, C.A. 1965. Silvics of western white pine (*Pinus monticola* Dougl.). Pp. 478-487 in H.A. Fowells (compiler), Silvics of forest trees of the United States. USDA Agric. Handbook 271. Washington, DC.

White, E.E. 1990. Chloroplast DNA in *Pinus monticola*. 2. Survey of within-species variability and detection of heteroplasmic individuals. Theor. Appl. Genet. 79: 251-255.

Woo, K.S., L. Fins and G.I. McDonald. 2001. Differences in needle morphology between blister rust resistant and susceptible western white pine stocks. Can. J. For. Res. 31: 1880-1886.

Yu, X.S., A.K.M. Ekramoddoullah and S. Misra. 1997. Cloning and constructing a cDNA database of genes involved in frost hardiness from western white pine needles. Plant Physiol. 114: 600-606.

Zavarin, E., K. Snajberk and L. Cool. 1990. Monoterpene variability of *Pinus monticola* wood. Biochem. Syst. Ecol. 18: 117-124.

Section 2.
Jack pine (*Pinus banksiana*)

1. Taxonomy and use

1.1. Taxonomy

The largest genus in the family Pinaceae, *Pinus* L., which consists of about 110 pine species, occurs naturally through much of the Northern Hemisphere, from the far north to the cooler montane tropics (Peterson, 1980; Richardson, 1998). Two subgenera are usually recognised: hard pines (generally with much resin, wood close-grained, sheath of a leaf fascicle persistent, two fibrovascular bundles per needle — the diploxylon pines); and soft, or white pines (generally little resin, wood coarse-grained, sheath sheds early, one fibrovascular bundle in a needle — the haploxylon pines). These subgenera are called respectively subg. *Pinus* and subg. *Strobus* (Little and Critchfield, 1969; Price *et al.,* 1998). Occasionally, one to about half the species (20 spp.) in subg. *Strobus* are classified instead in a variable subg. *Ducampopinus*.

Jack pine (*Pinus banksiana* Lamb.) and its close relative lodgepole pine (*Pinus contorta* Dougl. Ex Loud.) are in subg. *Pinus*, subsection *Contortae*, which is classified either in section *Trifoliis* or a larger section *Pinus* (Little and Critchfield, 1969; Price *et al.,* 1998). Additionally, subsect. *Contortae* usually includes Virginia pine (*P. virginiana*) and sand pine (*P. clausa*), which are in southeastern USA. Jack pine has two quite short (2-5 cm) stiff needles per fascicle (cluster) and lopsided (asymmetric) cones that curve toward the branch tip, and the cone scales often have a tiny prickle at each tip (Kral, 1993). Non-taxonomic ecological or biological variants of jack pine have been described, including dwarf, pendulous, and prostrate forms, having variegated needle colouration, and with unusual branching habits (Rudolph and Yeatman, 1982).

1.2. Uses

Jack pine is one of the most important commercial tree species in Canada and the Lake States of USA. Its wood is moderately hard and heavy, and relative to other softwoods, of intermediate strength (Eyre and LeBarron, 1944; Hosie, 1979). It can produce merchantable stands on sites often too poor and infertile for other tree species to thrive (Cayford and McRae, 1983). It has a number of commercial applications, including pulpwood, general construction timber, railway ties, poles, pilings, mine timbers and fuel (Rudolf, 1958; Hosie, 1979; Cayford and McRae, 1983; Law and Valade, 1994). Other applications include the extraction of essential oils for aromatic agents in products such as perfumes, cosmetics and cleaners (Marles *et al.,* 2000).

There were a number of traditional aboriginal uses of jack pine (Marles *et al.,* 2000), some of which are: inner bark and needles processed to yield poultice to treat wounds and frostbite; pitch chewed as a medicinal; dried cones used in tanning of hides; roots used to make baskets, and fish hooks made from knots. The wood was used for cabins, boat planks, fishnet floats and fuelwood. Though less effective than spruce (*Picea*) pitch, pine pitch could also be used for caulking.

2. Natural distribution and migrational history

2.1. Natural distribution

Jack pine is widespread through northern North America from the Atlantic coast to the low Rocky Mountains (Figure 1). With a mainly contiguous range, it is the most widely distributed pine species in Canada, and grows farther north than any other North American pine (Cayford *et al.,* 1967; Rudolph and Laidly, 1990). The natural range extends from southeastern Canada in Nova Scotia, Prince Edward Island and New Brunswick westward through much of south-central Québec, central Ontario, Manitoba, Saskatchewan and Alberta and the extreme northeast of British Columbia northward into the Northwest Territories (extending slightly into Nunavut). Toward the south, jack pine extends into the Lake States (eastern Minnesota, northern Wisconsin and Michigan) and to northern Illinois and Indiana. Its southern limit in the eastern USA is mainly in northern New York, Vermont and New Hampshire and central Maine, with an outlying population in eastern Pennsylvania (Rudolf, 1958; Rudolf, 1965; Kral, 1993). In the Lake States jack pine generally occurs at elevations between 300 m and 460 m above sea level; in the eastern portion of its range, it grows from near sea level to 850 m in elevation (Rudolf, 1965; Elias, 1980).

2.2. Centre of origin, evolution, and migrational history

The genus *Pinus* is ancient, believed to have originated in the early to mid-Mesozoic era about 180 million years ago, prior to continental separation in the Laurasian region that became eastern North America and western Europe (Burdon, 2002). Approximately 150 million years before the present (BP), *Pinus* diverged into hard pines (subg. *Pinus*) and soft pines (subg. *Strobus*) (Yeatman, 1967). Rapid evolution, speciation, and migration occurred during the Tertiary prior to cooling climatic conditions at its end (Mirov and Hasbrouck 1976). Lodgepole pine and jack pine might have evolved from a common progenitor into western and northern species during the cooling of the late Tertiary (Pliocene), or may not have diverged until the Pleistocene (Critchfield, 1984) — Dancik and Yeh (1983) estimated that they diverged between 485,000 and 565,000 years BP.

During the Pleistocene, jack pine retreated southward ahead of the advancing ice sheet. It was extirpated from northern regions prior to *Picea* (spruce), which could withstand the cooling temperatures longer. A main glacial refugium for this species was in the Appalachian Highlands (southeastern USA). Fossil evidence suggests that it also had at least two additional refugia in the American Midwest (Critchfield, 1984, 1985). Although it has been hypothesised that jack pine was able to persist alongside lodgepole pine in an unglaciated region between ice sheets in Alaska and the Yukon Valley (Mirov and Hasbrouck 1976), conclusive evidence for a western refugium was lacking until recently (Yeatman, 1967; Critchfield, 1985). Recent mitochondrial DNA minisatellite analysis, which identified three genetically distinct populations, has led to the inference that three distinct jack pine glacial refugia occurred: one west of the Appalachian mountain range, one east of these mountains, and a third in the unglaciated coastal region of eastern Canada (Godbout *et al., in press*). This work therefore concurs with fossil evidence and supports the theory of a western refugium.

With the retreat of the last Wisconsin glaciation beginning about 18,000 years BP, *Picea* species were the first coniferous postglacial colonisers, followed by northern migration of jack pine and lodgepole pine, which were in turn followed by red pine (*P. resinosa*) and eastern white pine (*P. strobus*). Jack pine expanded rapidly (350-500 m per year between 13,000-8,000 years BP) from the Appalachians to the Great Lakes and the Maritimes (Davis, 1976). It advanced into southern Ontario between 10,500 and 9,500 years BP, and underwent rapid expansion during a warm, dry period (Fuller, 1997). The last part of the Great Lakes area to be deglaciated was the north-central shore of Lake Superior (near Marathon, Ontario), which today has one of the most genetically distinctive populations in central Canada (Critchfield, 1985). Jack pine is estimated to have reached its northern limit in northwestern Québec

near Hudson Bay (at 55° latitude) about 3,000 years BP, some 4,000 years after the region was deglaciated (Desponts and Payette, 1993). The species is believed to have become established first in sporadic stands, from which it was able to colonise additional sites after fire.

Figure 1. Main natural distribution of jack pine in North America



Source: Courtesy of the Flora of North America Association

Populations from one Midwestern refugium migrated to Lower Michigan, while populations from the second refugium colonised Wisconsin and Minnesota (Critchfield, 1985). On prairie grasslands in north-central Minnesota, jack pine advance was curtailed by competition for water until about 5,000 years BP, when increased fire frequency favoured jack pine forestation (Almendinger, 1992).

Jack pine did not reach southern Manitoba until about 12,000 years BP (Jacobson *et al.,* 1987). The species expanded northwestward rapidly, extending into north-central Saskatchewan by about 8,000 years BP (McLeod and MacDonald, 1997). The rapid spread may have been in part due to the warm dry period with higher fire frequencies, favouring its regeneration. Further expansion northward continued at a reduced rate, coinciding with cooler temperatures and lower fire incidence. Jack pine reached its northwestern limit in the Northwest Territories (Upper Mackenzie River Valley) around 4,150 years BP (McLeod and MacDonald, 1997). Whereas the limiting factors in the western portion of the current range appear to be growing degree-days (>5ºC) and dominance of peat soils, the northern limit in the east appears to be caused by snow and the lack of sufficient fire (Despland and Houle, 1997; McLeod and MacDonald, 1997; Hofgaard *et al.,* 1999; Asselin *et al.,* 2003).

3. Reproductive biology

3.1. Reproductive bud differentiation

Jack pine is monoecious. It is a wind pollinated, cross-fertilising species, although some natural selfing can occur. Ovulate (female) strobili or cones ("flowers") are typically found on vigorous primary and secondary branches in the upper crown, and staminate (male) strobili on the less vigorous tertiary branches of the lower crown (Rudolph and Laidly, 1990). Like most other pines, jack pine has a 3-year reproduction cycle. Staminate cone primordia are initiated in early or mid-July, ovulate cone primordia in August (Curtis and Popham, 1972). Time of anthesis varies from year to year, ranging from mid-May to early June, and is generally synchronised with female cone receptivity (Rudolph and Yeatman, 1982). In southern Ontario, pollen shedding begins around the last week of May and continues for about a week (Ho, 1991). Ovulate cones begin to emerge from bud scales in mid-May. When fully emerged, margins of bracts are reflexed and cones at the peak of receptivity; this occurs about 25 May in Ontario (Ho, 1991; Roussy and Kevan, 2000). Following pollination, pollen tube growth and ovule development are initiated, but stop in mid-summer (Owens and Blake, 1985). They resume the following spring and fertilisation occurs about 13 months after pollination. Cones and seeds mature late in the growing season of the year of fertilisation (Rudolph and Laidly, 1990).

3.2. Natural seed production and dissemination

Jack pine is an early and prolific seed producer. Rudolph (1979b) observed ovulate strobili on plantation trees only 17 months in age. Typically, cone production begins at 5 to 10 years of age among open-grown trees and 10 to 25 years in closed stands; optimum seed production occurs between 40 to 90 years, varying with site and stand conditions (Roe, 1963; Rudolf, 1965). Annual seed production varies (*e.g.* Houle and Filion, 1993); some seed is usually produced each year (Rudolph and Laidly, 1990), with good cone crops occurring every 3 to 4 years (Eyre and LeBarron, 1944; Roe, 1963). A cone may produce 17 to 40 filled seeds (Roe, 1963; Cayford *et al.,* 1967; Jeffers, 1972; Houle and Filion, 1993; Greene and Johnson, 1999).

Over most of its range, jack pine bears serotinous cones, an adaptation that can result in significant quantities of viable seed dispersal following fire. In the absence of fire, cones may remain closed for more than 25 years (Roe, 1963). Seeds within closed cones maintain high viability for at least 5 years; even after 20 years, average germination may reach 50% (Rudolf, 1965; Cayford and McRae, 1983). In the southern part of its range, jack pine produces non-serotinous cones, which soon open without fire (Ahlgren, 1974). Trees may have 10 or more annual cone crop cohorts (Greene and Johnson, 1999; Greene *et al.,* 1999). Consequently, jack pine can maintain a substantial aerial seedbank; estimates for well-stocked stands range from 1 million to 4 million seeds per ha (Rudolf, 1965; Greene *et al.,* 1999). Fires of suitable intensity and duration cause the cones to open and release seed, while leaving most seed undamaged and viable (de Groot *et al.,* 2004). Seed may be released within the first few days following fire (Eyre and LeBarron, 1944); the majority of seed is released within 3 or 4 years (Greene and Johnson, 1999), often leading to post-fire stands that are even-aged. The extent of early regeneration and establishment on a site may correlate to the pre-burn basal area, reflecting the size of the aerial seed bank (Greene and Johnson, 1999; Arseneault and Sirois, 2004).

3.3. Natural regeneration

3.3.1. Seedling regeneration

Jack pine seed does not require stratification (Yeatman, 1984). It has epigeal (aboveground) germination. Favourable seedbeds include mineral soil, decomposed organic layers less than a few centimetres thick, and burned or scarified duff (Rudolf, 1965; Cayford and McRae, 1983; Greene

et al., 1999). Feather mosses and herbaceous, grass and shrub litter make poor seedbeds (Cayford, 1963a). Undisturbed surface humus may hinder seed germination and seedling survival (Chrosciewicz, 1970). Partial shade may enhance germination and early establishment (Eyre and LeBarron, 1944; Rudolf, 1958; Cayford, 1963b); however, full sunlight is subsequently required for optimal growth and survival (Rudolf, 1965). Most germination occurs promptly following seed dispersal, if temperature and moisture conditions are suitable (Cayford and McRae, 1983). Establishment is better when seed dispersal is in spring and early summer rather than autumn (Rudolf, 1958; Chrosciewicz, 1988b). A proportion of seed may not germinate until one or two growing seasons after dispersal (Ahlgren, 1959; Thomas and Wein, 1985); St-Pierre *et al.,* (1992) found that 95% of seedlings were established within 3-years following fire.

Forest fire may enhance seedbed quality by reducing accumulated organic layers, reducing plant competition and pest populations, and providing nutrients (Cayford, 1963b). Prescribed burns that reduce surface litter and raw humus depth, while exposing mineral soil and reducing aerial parts of competing vegetation, improve stocking and subsequent height growth (Chrosciewicz, 1970, 1988b). Scarification to expose mineral soil and reduce the thickness of litter following harvesting may enhance germination (Cayford, 1959). The level of rainfall can affect the quality of the seedbed in a manner that varies with soil type and level of the water table (Rudolf, 1958; Chrosciewicz, 1988b); early seedling height growth may be affected by vegetation competition and the soil moisture regime (Chrosciewicz, 1970). The importance of an appropriate seedbed becomes more pronounced when weather conditions are less favourable for germination and early growth (Benzie, 1977). Early seedling mortality due to heat and drought can be substantial, particularly on dry sites, although mitigated by shade (Cayford *et al.,* 1967).

3.3.2. Vegetative propagation

Jack pine does not naturally reproduce through vegetative propagation (Rudolf, 1958).

3.4. Mating system and gene flow

Jack pine is monoeceous, with a mixed mating system. While it is mainly outcrossing, self-pollination also occurs. Selfing rates of between 7% and 12% have been reported (Sittmann and Tyson, 1971; Rudolph 1979a; Cheliak *et al.,* 1985). Most of the genetic variation resides within populations (regardless of the distance between sampled populations). Outcrossing rate estimates generally range from 88 to 98% (Dancik and Yeh, 1983; Danzmann and Buchert, 1983; Cheliak *et al.,* 1985; Snyder *et al.,* 1985; Ross and Hawkins, 1986; Misenti and DeHayes, 1988; Fu *et al.,* 1992; Gauthier *et al.,* 1992; Godt *et al.,* 2001; Saenz-Romero *et al.,* 2001).

Very weak patterns of family substructuring have been observed in jack pine stands (Cheliak *et al.,* 1985; Xie and Knowles, 1991; Saenz-Romero *et al.,* 2001). Dong and Wagner (1994) found that maternally inherited mitochondrial DNA showed higher levels of population subdivision than paternally inherited chloroplast DNA. No differences in isozyme variation were detected between natural and plantation stands (Knowles, 1985).

Jack pine seed and pollen are windborne. Seed does not disperse beyond about 30 m from the parent tree (Rudolf, 1965). However, gene flow through pollen dissemination is extensive. A jack pine pollen grain is only about 50 µm wide (including the two air-bladders) (Di Giovanni *et al.,* 1995), and is therefore able to travel long distances. Di Giovanni *et al.,* (1996) obtained samples of its pollen 300 m above the ground; in a steady wind of 5 m per sec, they estimated that the pollen could drift about 60 km. Saenz-Romero *et al.,* (2001) estimated gene flow to be more than 11 migrants per year whereas Godt *et al.,* (2001) obtained a rate of 16.9 migrants per year, both thus indicating that extensive migration has a large influence on the species' genetic structure.

Xie and Knowles (1991) suggested that short seed dispersal distances may cause small-scale non-random genetic spatial patterns but these would not occur over a large scale. They suggested that high gene flow resulting from long-distance pollen migration overwhelms forces (such as genetic drift) that promote subpopulation differences, and causes the lack of variation observed between populations.

4. Hybridisation

Jack pine and lodgepole pine (*P. contorta* subsp. *latifolia*) share a sympatric region in Canada in central and northwestern Alberta to the Northwest Territories. Genetic remnants of lodgepole pine within the jack pine population in Saskatchewan have also been detected (Rudolph and Yeatman, 1982; Dong and Wagner, 1993). Where the species coexist, natural hybridisation occurs. More widespread hybridisation is prevented by phenological differences in female strobili receptivity and pollen shed in these species, with jack pine flowering 2 to 3 weeks earlier (Critchfield, 1985). Some artificial F_1 hybrids have high levels of pollen abortion, but F_1 to F_3 hybrids produced some sound seed (Critchfield, 1980). Zavarin *et al.,* (1969) described the introgression of jack pine genes into lodgepole pine stands 150 km south of the hybrid zone, and lodgepole pine genes into jack pine stands 150 km north of the closest lodgepole pine stand. Yet the genetic distance between jack pine and lodgepole pine populations in Alberta based on allozymes averages 20 times greater than among populations within each species (Dancik and Yeh, 1983). In the sympatric region, jack pine is xerophytic, and often found on well-drained, sandy sites, whereas lodgepole pine is mesophytic, tolerant of heavier, wetter clay soils, and more typical at higher elevations; hybrids commonly occupy intermediate sites (Wheeler and Guries, 1987; Yang *et al.,* 1999).

Artificial hybrids reportedly have been made between jack pine and Virginia pine and loblolly pine respectively. Artificial hybridisation has also been reported with Japanese black pine (*P. thunbergii*). Nonetheless, the only artificial hybrid sufficiently verified is with lodgepole pine (Rudolph and Yeatman, 1982). Crossing between members of different *Pinus* subsections typically does not occur, because of genetic barriers (Critchfield, 1975). Artificial crosses and backcrosses between jack pine and lodgepole pine were carried out in many early programs in an attempt to combine the fast growth and relative pest resistance of jack pine with the stem form of lodgepole pine.

Even though lodgepole pine is more genetically variable than jack pine (Dancik and Yeh, 1983), it is more susceptible to sweetfern rust (*Cronartium comptoniae*) and western gall rust (*Endocronartium harknessii*) and eastern gall rust (*Cronartium quercuum*) (Anderson and Anderson, 1965; Yeatman, 1974, Yang *et al.,* 1998, 1999). Unfortunately, the pines' hybrids were also more susceptible to western gall rust than was jack pine (Yang *et al.,* 1999). As lodgepole pine resistance to western gall rust, needlecast (*Davisomycella ampla*), stalactiform blister rust (*Cronartium coleosporioides*), and Sequoia pitch moth (*Synanthedon sequoiae*) increases clinally with proximity to the jack pine range (Wu *et al.,* 1996; Wu and Ying, 1998), it appears that introgressed jack pine genes are conferring resistance to lodgepole pine. However, other explanations can be proposed for the clinal trends in lodgepole pine resistance. Yang *et al.,* (1997) have questioned whether the introgression interpretation is valid for western gall rust; neither study sampled non-hybrid jack pines.

Numerous morphological traits such as needle length, cone characteristics and turpentine composition appear intermediate in hybrids (Moss, 1949; Mirov, 1956; Keng and Little, 1961; Zavarin *et al.,* 1969; Rudolph and Yeatman, 1982). Rudolph and Nienstaedt (1962) found that hybrid resistance to winter injury was intermediate between the hardy jack pine and less hardy lodgepole pine. Whereas early hybrid growth and survival is considered generally to be intermediate to that of jack pine and lodgepole pine (Lotan, 1967; Yeatman and Holst, 1972; Garrett, 1979; Yang *et al.,* 1999), little difference in performance was noted by age 15 to 20, which led Rehfeldt and Lotan (1970) to conclude that lodgepole pine × jack pine hybrid breeding programs were not warranted.

Hybridisation, backcrossing and introgression have been characterised by allozyme variation (Wheeler and Guries, 1987; Dancik and Yeh, 1983) and randomly amplified polymorphic DNA (RAPD) variation (Ye *et al.,* 2002). Paternally inherited chloroplast DNA exhibits atypical, novel variants in the zone of sympatry (Wagner *et al.,* 1987, 1988, 1991; Govindaraju *et al.,* 1988). Maternally inherited mitochondrial DNA distinguished between each species and their hybrids, and was found much less variable in jack pine than in lodgepole pine (Dong and Wagner, 1993).

5. Genetics

5.1. Cytology

The diploid (2*n*) chromosome number of jack pine (and all members of the genus *Pinus*) is 24. Saylor (1972, as summarised by Rudolph and Yeatman, 1982) found that 11 of the chromosomes had median centromeres, but the 12th and shortest chromosome was heterobrachial; the chromosomes varied in length by a factor of 0.6. The diploid DNA genome size of jack pine has been estimated to be 29.8 picograms (Rake *et al.,* 1980), with variability per cell and among genetic families (Miksche, 1968; Wyman *et al.,* 1997).

5.2. Inbreeding depression

Inbreeding depression caused by self-pollination has been expressed by lower seed set, mortality, abnormal germination, abnormal phenotypes, chlorophyll deficiencies, lower water-use efficiency, lower growth rates, delay in initiation of flowering, and lower fecundity (Fowler, 1965a, 1965b; Rudolph 1966a, 1981b; Blake and Yeatman, 1989). Rudolph (1981a) observed inbreeding depression in tree height between 18 to 24% in selfed S_2 progeny.

Fowler (1965b) observed about 13% selfing in the upper crown and 26% in the lower crown of seed orchard trees. However, many selfed progeny would not be expected to survive; Sittmann and Tyson (1971) estimated an inbreeding rate due to selfing of 5% per generation for a population in Hardy-Weinberg equilibrium. As jack pine retains its serotinous cones for years, comparisons can be made between seed of the same tree produced in different years (Teich, 1970). Higher rates of selfed seed are found in the latest seed (Cheliak *et al.,* 1985; Snyder *et al.,* 1985); it appears that selection against inbred seed is occurring at a linear rate, with a loss of viability of selfed seed.

5.3. Genetic variation

5.3.1. Population-level variation

Jack pine provenance testing has been carried out on a regional and a rangewide basis, and included growth chamber, greenhouse, nursery, and field experiments. Besides being tested throughout its natural range in Canada and USA, its seed has been distributed to Great Britain, The Netherlands, New Zealand and Japan for testing (Rudolph and Yeatman, 1982). Numerous investigations have described a pattern of clinal variation, usually associated with temperature (growing degree-days) and photoperiod (latitudinal) effects, particularly where cold temperatures and growing degree-days are not limiting. When grown in a common environment, northern provenances which originate in areas with colder temperatures and fewer growing degree-days are typically smaller in height, diameter and volume than southern sources (Schantz-Hansen and Jensen, 1952; Giertych and Farrar, 1962; Sweet and Thulin, 1963; Yeatman, 1974; Canavera, 1975; Skeates, 1976; Hyun, 1979; Jeffers and Jensen, 1980; Rudolph and Yeatman, 1982; Magnussen and Yeatman, 1988b; Bolstad *et al.,* 1991; van Niejenhuis and Parker, 1996). This trend was observed from the early seedling stage through to age 20, although differences lessened with age (Yeatman, 1974).

Jeffers and Jensen (1980) found more variability in volume among sources than height, diameter or survival; volume was not correlated with growing degree-days, unlike the other traits. Magnussen and Yeatman (1979) observed a larger amount of within-population than between-population variation for height. Survival was correlated to the similarity in climatic conditions between source origin and test site (Jeffers and Jensen, 1980). Higher mortality resulted in less competition, and subsequently increased growth rates (Jeffers and Jensen, 1980).

Seedling leaf, root, and total dry weights were also found to vary in relation to growing degree-days of the source (Giertych and Farrar, 1962). Aboveground biomass was strongly related to water availability, except for provenances from warmer climates suspected of being more tolerant to water stress or having more efficient water usage (Strong and Grigal, 1987). There was greater variability between provenances on more adverse sites. Dry weights of 4-month-old seedlings correlated with height after 4 years (Yeatman and Holst, 1967).

Under environment chamber conditions, seed source differences between jack pine seedlings were most readily observed under short photoperiods (Mergen *et al.,* 1967). Northern provenances were more responsive to changes in photoperiod than southern sources (Giertych and Farrar, 1962; Yeatman, 1974). In field trials, more variability between populations in height growth was observed at milder test sites (Jeffers and Jensen, 1980). Taller provenances were found to retain higher photosynthetic rates into autumn than slower-growing sources (Logan, 1971). However, no provenance differences were detected in stem respiration (Lavigne, 1996).

Southernmost provenances typically begin flowering at a younger age (Sweet and Thulin, 1963). Western provenances bear smaller cones with fewer seeds (Jeffers, 1972; Schoenike, 1976). Most of the total variation in cone and seed traits resided between populations. Climatic variables were related to chemical components in embryo and megagametophyte, although environmental preconditioning may have influenced nutrient levels in the megagametophyte (Durzan and Chalupa, 1968). Maternal effects of seed weight have been observed on germinants, but diminished by the time seedlings were 3 months old (Yeatman, 1974). No differences between sources were noted in timing or rate of germination (Yeatman, 1966). Seed weight is highest in populations from regions with longer and warmer growing seasons.

Southern provenances are slower to flush in spring, set bud at the end of the growing season, and form secondary needles than northern provenances (Yeatman, 1974). Budburst is strongly associated with colder mean January temperatures (Steiner, 1979). Coastal populations burst bud later than more continental sources. Significant provenance differences have been observed in lammas growth (second, late-season terminal shoot extension) and proleptic growth (second, late-season extension of the lateral buds at the base of the terminal bud) (Rudolph 1964). Cessation of cambial activity was found to be under strong genetic control, but there was less control over initiation of cambial activity (Kennedy, 1971). Provenance differences have been noted in foliar nitrogen, phosphorus, potassium and calcium (Mergen and Worrall, 1965; Giertych and Farrar, 1962; Strong and Grigal, 1987). Foliar nitrogen content correlated with growing degree-days (Giertych and Farrar, 1962), with northwestern provenances having the highest foliar nitrogen (Mergen and Worrall, 1965). Strong and Grigal (1987) hypothesised that foliar macronutrient provenance differences were slight because jack pine is efficient in using available nutrients.

Northern provenances develop winter foliage colouration more rapidly and attain a deeper hue than southern provenances (Canavera, 1975; Rudolph and Yeatman, 1982; vanNiejenhuis and Parker, 1996). The purpling is attributed to at least five anthocyanin pigments, which are produced after the first autumn exposure to freezing temperatures (Nozzolillo *et al.,* 2002). Young seedling foliage turns purplish in autumn, but as seedlings age, winter colouration becomes a yellow-bronze. Northern sources are older than southern sources before making the transition between purple and bronze foliage (Canavera and Wright, 1973). Northern provenances develop cold hardiness earlier in autumn (Yeatman, 1974). Sources from warmer climates have wider annual rings, wider earlywood and latewood rings,

and slightly lower specific gravity (Kennedy, 1971). Provenance differences were observed in both tracheid length and wood specific gravity, with a larger between-stand than within-stand component (King, 1968). Northern Canadian provenances and sources from Nova Scotia had the slowest growth, shortest tracheid length and highest specific gravity, whereas U.S. populations from Michigan, Wisconsin and Minnesota had the fastest growth, longest tracheid length and lowest specific gravity. Crown width and bark thickness are negatively correlated with latitude (Hyun, 1979). One Atlantic coast source from Maine (USA) was noticeably prostrate by age 4, similar to its parental types, when grown in Minnesota (Schantz-Hansen and Jensen, 1952).

Numerous studies have reported jack pine resistance at the population level to various pests (Yeatman and Teich, 1969). Southern sources growing on northern test sites had a higher incidence of scleroderris canker (*Gremmeniella abietina)*, with best resistance from Québec sources (Yeatman and Morgenstern, 1979). Taller trees were found to have a higher incidence of eastern gall rust infection (Bolstad *et al.,* 1991). However, in Minnesota southern Lake States sources were less susceptible to eastern gall rust than northern sources (King, 1971; Jeffers and Jensen, 1980). Other pests for which resistance has been reported include white pine weevil (*Pissodes strobi*) (Arend *et al,*. 1961; King, 1971), bark beetles (*Pityophthorus* spp), redheaded pine sawfly (*Neodiprion lecontei*) (Arend *et al.,* 1961; Schantz-Hansen and Jensen, 1952), eastern pine shoot borer (*Eucosma gloriola*) (King, 1971) and needlecast (*Davisomycella (Hypodermella) ampla*) (King and Nienstaedt, 1965).

Most studies involving interprovenance crosses have found that these intraspecific hybrids are approximately intermediate to the parental types. Traits showing this include height, stem characteristics, crown characteristics (Magnussen and Yeatman, 1988b; Bolstad *et al.,* 1991), survival (Magnussen and Yeatman, 1988a), and shoot extension periodicity (Magnussen and Yeatman, 1979). However, Magnussen and Yeatman (1988a) observed significant heterosis (departure from midparent value) in height at all test sites except the northernmost, where provenance differences were not significant. No significant reciprocal cross effects have been found (Magnussen and Yeatman, 1988a; Bolstad *et al,*. 1991).

Provenance by environment interactions have been observed in many traits of jack pine. Source by temperature and source by photoperiod interactions in number of primary needles produced were noted in a controlled-environment seedling experiment (Mergen *et al.,* 1967). Provenance by site interactions occurred in aboveground total biomass production of six provenances grown at eight sites throughout the Lake States (Strong and Grigal, 1987). While no provenance by site interaction was noted in height at two test sites in New Zealand (Sweet and Thulin, 1963), many studies located within jack pine's natural range have described strong provenance by site interactions for height, diameter, volume, and survival from seedling stage through to age 20 (Jeffers and Jensen, 1980). Northern and southern provenances appear to contribute more to these source by environment interactions than midlatitude provenances (Morgenstern and Teich, 1969; Jeffers and Jensen, 1980; Magnussen and Yeatman, 1988b), perhaps due to the greater distance between origin and planting site for those populations (Morgenstern and Teich, 1969).

Age 3 was considered too young for selection of wood quality traits, in particular specific gravity (King, 1968). Provenance trials have indicated that age-5 height is not reliable for predicting height at age 20 (Yeatman, 1974; Jeffers and Jensen, 1980). However, by age 10 to 15, height effectively predicted growth at age 20, and could reliably be used to develop seed source recommendations (Yeatman, 1974; Jeffers and Jensen, 1980). Magnussen and Yeatman (1988b) suggested that height after 8 to 10 years could be used as a reliable indicator of later performance when testing was carried out on favourable sites, but on adverse sites, decisions regarding seed source selections should be delayed until the tests are older.

5.3.2. Within-population variation

High levels of genetic variation in jack pine growth have been documented in numerous studies. Table 1 lists a sampling of individual and family heritabilities reported for height growth from sites in the central to eastern range (Manitoba [MB], Ontario [ON], Wisconsin [WI] and New Brunswick [NB]).

Table 1. Examples of jack pine individual (h^2_i) and half-sib family (h^2_f) heritabilities for height.

Reference	location	# families	# sites	age	h^2_i	h^2_f
Klein, 1989b	MB	216	3	10	0.1	0.49
Klein, 1995	MB	215	3	20	0.34	0.68
Magnussen & Yeatman, 1990	ON	100	3	6	0.13	0.42
" "	"	"	"	14	0.18	0.48
Morris *et al.,* 1992	ON	369	1	3	0.17	0.26
Riemenschneider, 1988	WI	102	1	7	0.2	–
Adams & Morgenstern, 1991	NB	104	4	7	0.17	0.74
Park *et al.,* 1989	NB	162	4	10	0.26	0.81

Estimates of the amount of total variation due to family are below 20% (Canavera, 1975; Klein, 1989b; Park *et al,.* 1989). Genetic gain is typically low; height gains of seedlings aged 2 or 3 range from 0.4 to 5.1% (Canavera, 1975; Rudolph *et al.,* 1989; Adams and Morgenstern, 1991). At age 20, Klein (1995) estimated a genetic gain of 5.7% for height; Simpson and Steel (1995) reported a height gain of 3.2% in a seed orchard after 14 years and three roguings.

Riemenschneider (1988) proposed that early selection based on young seedling performance might be efficient in improving growth over a rotation. Carter *et al.,* (1990) found that accelerated-grown seedlings that underwent two growing cycles were adequate for roguing out the poorest families prior to installation of field tests, although some misclassification in family ranking occurred in comparison to height at age 7. Genetic correlation for height between age 6 and age 14 was estimated at 0.71 (Magnussen and Yeatman, 1990). Jeffers and Jensen (1980) found that height at age 10 reliably predicted height at age 20.

Clonal and/or family variation was observed for a number of cone and seed production traits, including cone volume, number of empty seeds, and seed potential (Todhunter and Polk, 1981; de Groot and Schnekenburger, 1996). While variation has been observed in cotyledon number and length and timing of budset (Saenz-Romero and Guries, 2002), lammas growth was not related to height and did not differ between families (Canavera, 1975). The latter study also found no family differences in the age when flowering first occurred.

Maley and Parker (1993) intensively studied the region in northwestern Ontario around Lake Nipigon (north of Lake Superior), which is known for its east-west discontinuity in the clinal variation of many jack pine traits. Van Niejenhuis and Parker (1996) noted that irregularities in clinal patterns from this area correspond to irregularities in local climate patterns. Most of the variation in cone and needle traits was within stand or within tree, not between stands. Results were inconclusive in differentiating between the hypothesis that separate lineages migrated in opposite directions around Lake Superior post-glaciation, and evidence that the patterns are caused by adaptation to an abruptly changing climate within the region.

As observed in many species, wood quality traits of jack pine appear to be more highly heritable than growth traits. Family heritability estimates for wood density of trees aged 5 to 20 range from 0.40 to 0.73, and individual heritabilities between 0.31 and 0.93 (Okwuagwu and Guries, 1980; Ernst *et al.,*

1983; Villeneuve *et al.,* 1987a, 1987b; Park *et al.,* 1989; Magnussen and Keith, 1990; Klein, 1995). Family heritability for tracheid length is also high (0.50) (Okwuagwu and Guries, 1980). Stem straightness is also highly heritable, with an estimated h^2_i of about 0.3 (Magnussen, 1990; Klein, 1995). Conversely, while the number of leaders was variable, there was no genetic component to the variability (Morris *et al.,* 1992). Most of these estimates were based on single-site analyses, and thus possibly inflated, but it is evident that wood quality traits are highly heritable.

Relative density decreased from the pith to between age 9 to 12, then rapidly increased to age 15, after which increases were more gradual (Villeneuve *et al.,* 1987b). It has been suggested that wood density could be selected for by age 6 or 7 (Villeneuve *et al.,* 1987b). The Pilodyn tester was considered reliable enough for low-intensity selection to rogue out families with the lowest wood density (Villeneuve *et al.,* 1987a), but not recommended for selection of the best genotypes (Zhang, 1995).

General combining ability, but not specific combining ability, was significant for both juvenile wood specific gravity and tracheid length (Okwuagwu and Guries, 1980). Wood density and stem taper were found to have low phenotypic variation (as expressed by the coefficient of variation), compared to high phenotypic variation observed in stem volume, percent heartwood, and dry fiber weight (Magnussen and Keith, 1990; Zhang and Chui, 1996). Most of the phenotypic variation in wood density resides within families, with between 7% and 11% due to family differences (Villeneuve *et al.,* 1987b; Park *et al.,* 1989; Magnussen and Keith, 1990; Zhang and Chui, 1996).

Jack pine growth traits are negatively correlated with wood quality traits, in particular stem straightness, and branch angle, length and diameter (Park *et al.,* 1989; Adams and Morgenstern, 1991; Morris *et al.,* 1992). Park *et al.,* (1989) described a negative relationship between growth and wood density. However, others have found that stem volume and wood density were either not correlated or positively correlated (Magnussen and Keith, 1990; Zhang, 1995; Zhang and Chui, 1996), implying that breeding programs based on selection for growth would not necessarily cause a reduction in wood density. Stand spacing also affects growth and wood quality, with wide spacing producing poor stem and branch form, and dense spacing causing reduced growth due to crown closure and suppression (Magnussen and Yeatman, 1987).

Isozyme variability in jack pine has been well documented (*e.g.* Dancik and Yeh, 1983; Danzmann and Buchert, 1983; Cheliak *et al.,* 1985; Snyder *et al.,* 1985; Wheeler and Guries, 1987; Xie and Knowles, 1991; Fu *et al.,* 1992; Gauthier *et al.,* 1992; Godt *et al.,* 2001; Saenz-Romero *et al.,* 2001). Variability has also been observed in RAPD (Nkongolo and Gratton, 2001; Ye *et al.,* 2002) and sequence-tagged-site (STS) (Perry and Bousquet, 1998) markers, chloroplast DNA (Wagner *et al.,* 1987, 1988, 1991; Govindaraju *et al.,* 1988), and mitochondrial DNA (Dong and Wagner, 1993). Variability in the amount of DNA per cell has been observed (Miksche, 1968), with superior families having less embryo and megagametophyte nuclear DNA than inferior families (Wyman *et al.,* 1997). Superior families were also more amenable to producing viable plants from *in vitro* culture (Briand *et al.,* 1998). Gene transfer in jack pine has been accomplished using electroporation (Tautorus *et al.,* 1989), microprojectile bombardment (Hay *et al.,* 1994), and *Agrobacterium rhizogenes* (McAfee *et al.,* 1993).

Family differences have been observed in water-use efficiency (Cantin *et al.,* 1997) and carbon isotope discrimination (Zhang and Cregg, 2096). Jack pine is more tolerant of soil salinity and alkalinity than lodgepole pine, white spruce (*Picea glauca*), poplar (*Populus*) and alder (*Alnus*). Genotypic differences within species were noted in response to salinity by Khasa *et al.,* (2002), who suggested developing breeding programs for salt-tolerant genotypes for reclamation of oil sand deposits.

5.3.3. Resistance to pests

Differences between sources in resistance to white pine weevil have been reported (Batzer, 1961, 1962; King, 1971; Hodson *et al.,* 1982; de Groot and Schnekenburger, 1999). Batzer (1962) and de Groot

and Schnekenburger (1999) observed that local sources may be the most weevil-resistant, best-adapted populations. King (1971) and Hodson *et al.,* (1982) found that the tallest trees were the most heavily weeviled.

Northern jack pine populations were more susceptible to the eastern pine shoot borer (King, 1971; Jeffers, 1978; Hodson *et al.,* 1982, 1986). Jeffers (1978) observed that incidence was correlated with length of the terminal shoot during the period of borer oviposition in May, with longest terminals having highest incidence and shortest terminals the lowest. Source variability was also described for resistance to the northern pitch twig moth, but was not related to latitude (Hodson *et al.,* 1982, 1986).

Northern jack pine populations are more susceptible to eastern gall rust than southern populations (King, 1971; Hodson *et al.,* 1982, 1986). King (1971) proposed that southern populations may have been subjected to more intense infestations and so developed resistance. As eastern gall rust is morphologically indistinguishable from western gall rust, King (1971) proposed that resistance may be conferred against both rusts.

Jack pine needlecast fungus ranges from Wisconsin to Nova Scotia. Source differences were found in susceptibility to it, and consistent over years and across sites, when tested in the Lake States (King and Nienstaedt, 1965). The most resistant jack pine populations were from Lower Michigan, the least resistant from northeastern Minnesota.

Jerome and Ford (2002) studied the relationship between *Arceuthobium americanum* (lodgepole pine dwarf mistletoe), which is primarily western in range, and its two host species, lodgepole pine and jack pine. There were three genetic races of the parasite, but genetic distances revealed that hosts were divided into only two groups: one being lodgepole pine, and the other jack pine and its hybrids with lodgepole pine. The mistletoe strain on jack pine had twice as much population differentiation as that of the host.

King (1971) proposed that heavy infestations of pests may not affect tree height, as laterals quickly assume dominance if the terminal shoot is damaged. However, Hodson *et al.,* (1982) observed that eastern gall rust, pine shoot borer, white pine weevil and redheaded pine sawfly infestations all caused height decreases by age 15.

6. Tree growth and phenology

Jack pine is a relatively small tree, often up to 20 m tall with a dbh of 30 cm, occasionally reaching 25 m tall with a dbh of 35 cm. The bark is thin, reddish-brown to gray, becoming dark brown and flaky with age. Eyre and LeBarron (1944) noted the following factors as contributors to optimum early growth: (1) full sunlight; (2) fertile, well-drained but moist soils; (3) moderate summer temperatures; and (4) freedom from pests and competition. Young seedlings are very sensitive to shade and root competition. Compared to associated conifer species in the Lake States, juvenile growth rates are high (Eyre and LeBarron, 1944). With the exception of tamarack (*Larix laricina*), growth rate for the first 20 years is generally greater than for any other conifer in its natural range (Rudolf, 1965). In 5-year-old New Brunswick plantations, average height ranged from 1.2 to 1.4 m (Barteaux and Bailey, 1986). In Manitoba, 5-year-old seedlings averaged 0.5 to 1.2 m; growth was poorest on dry sites, better on fresh sites, and best on moist sites (Cayford, 1963a).

Shoot growth begins from late April to early May at sites in Minnesota, Michigan and Ontario, and is largely completed in 61 to 68 days (Rudolph and Laidly, 1990). In Wisconsin shoot elongation begins in the first week of May and is complete by mid-July; differentiation of lateral bud primordia occurs from early July to September (Cecich, 1983b). First year seedlings may exhibit continuous height growth into autumn as long as the temperature and moisture conditions are favourable (Eyre and LeBarron, 1944).

Jack pine may develop a taproot that is maintained into maturity. Where a distinct taproot is lacking, lateral roots may turn and grow downward in the proximity of other trees (Rudolf, 1958). On deep soils, roots may reach a depth of 270 cm or more, but more often the bulk of roots occur within the upper 46 cm (Rudolph and Laidly, 1990).

Jack pine is generally rather short-lived, with individuals living up to 180 to 200 years. Stands may survive to 100 years (Benzie, 1977), although they usually begin to disintegrate after 80 years on good sites and 60 years on poor sites (Rudolf, 1965). In Ontario, jack pine grows rapidly for the first 40 years, then shows growth reduction at rates that vary with stand and site conditions (Galloway, 1986). Height and diameter growth of stands vary with a number of factors, including soil moisture regime, soil texture and petography, and regional macroclimate; in northern Ontario, site indices decreased from the mid-humid warm-boreal climate (Hills Site Region 4E) to the dry-humid mid-boreal climate (Site Region 3W) (Chrosciewicz, 1963). The best growth and development in Canada is achieved in a wide area to the north and west of Lake Superior (Hosie, 1979).

Jack pine exhibits an intermediate self-pruning capacity in dense stands. On better sites, stands can undergo considerable mortality during intermediate stages of development because of natural thinning (Galloway, 1986; Kenkel *et al.,* 1997). On poorer sites, however, natural thinning may be slow (Benzie, 1977). Open-grown trees and those on poor sites may be short and shrub-like, developing undesirable branch and form characteristics (Eyre and LeBarron, 1944; Galloway, 1986). In well-stocked stands, trees develop a narrow crown that may cover 30 to 45% of the stem (Rudolf, 1965).

Under favourable conditions, jack pine may exhibit both lammas and proleptic growth. Rudolph (1964) found that trees exhibiting lammas growth had a longer growing period and greater total growth than individuals that exhibited normal growth, although the resulting shorter internodes adversely affected wood quality. Lammas growth may be more susceptible to autumn frost injury.

7. Ecology

7.1. Habitat

7.1.1. Climate

Across its broad range, jack pine tolerates a wide range of climatic conditions. Though the eastern portion of its range has a maritime climate, most of the range is inland and continental, with warm to cool summers, very cold winters, and low rainfall (Rudolf, 1965). Rudolph and Laidly (1990) have described the climate: Average temperatures range from -29ºC to -4ºC in January and 13ºC to 22ºC in July. Average annual minimum and maximum temperatures range from -46ºC to -21ºC and 29ºC to 38ºC respectively, with annual mean temperatures ranging from -5ºC to 4ºC. Average total annual precipitation ranges from 250 to 1,400 mm, with 380 to 890 mm more common. Summer droughts are common in the Lake States and western portion of the range (Rudolf, 1958).

The northern limit of the species' range closely follows the 29ºC mean annual isotherm (Rudolph and Laidly, 1990; Despland and Houle, 1997), extending into the permafrost zone in the northwest (Rudolf, 1958). The frost-free period typically averages 80 to 120 days, with extremes ranging from 50 to 173 days (Cayford *et al.,* 1967; Rudolph and Laidly, 1990). The date of the last killing spring frost ranges from 30 April to 1 July, and of the first killing autumn frost from 10 August to 20 October (Rudolf, 1958).

7.1.2. Soils and site types

Across its range, jack pine occurs on a variety of soils and site types, although most characteristically on sandy soils (Spodsols or Entisols) (Rudolph and Laidly, 1990). Typical sites include dry sand plains

developed on glacial outwash, morainic, aeolian and lacustrine deposits (Cayford and McRae, 1983). It is also found on fresh to moist sands, on tills, and thin soils overlying rock outcrops. In Ontario it can be found on glacio-fluvial plains, eskers, dunes and kames of sandy acidic soil, and occasionally on lowland sands and clays (Chrosciewicz, 1963). In the Lake States, it typically occurs on acidic sand plains of glacial-outwash origin, with low moisture and fertility, and a level or gently rolling topography (Eyre and LeBarron, 1944; Rudolf, 1963). In the eastern to central range, jack pine is also found on the thin moderately fertile rock-outcrop soils that overlay the Canadian (or Precambrian) Shield (sometimes called the Laurentian Plateau). In northwestern Canada, it may also grow on morainic hills (Rudolf, 1958).

The more productive sites in Ontario include silty sands, loamy sands and loams (Chrosciewicz, 1963), although competition from other species may limit its occupation. Productivity studies carried out in Québec, Ontario, Manitoba and Saskatchewan show that jack pine generally achieves best growth on fresh to somewhat moist upland till sites of fine sand to clay texture, as well as on moist sands (Cayford *et al.,* 1967). Best growth in the Lake States occurs in Minnesota, where despite lower rainfall than other states of the region, soils are generally more fertile (Eyre and LeBarron, 1944). Poorer sites include dry, weakly podzolised sands and wet poorly drained soils (Cayford *et al.,* 1967). Because of its modest moisture and nutrient requirements, jack pine can grow on sites too dry, shallow or infertile for competing tree species to survive.

7.2. Synecology and associated species

Jack pine typically establishes after fire, often forming pure, even-aged stands. Frequently, however, it is associated with other tree species. Throughout the boreal forest, it commonly occurs with black spruce (*Picea mariana*) on moist to fresh sites, and with trembling or quaking aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*) on silty sands, loamy sands and loams; and less commonly with balsam fir (*Abies balsamea*) and white spruce (*P. glauca*) (Chrosciewicz, 1963; Rudolph and Laidly, 1990). In the Great Lakes–St. Lawrence Forest Region, it also occurs with northern pin oak (*Quercus ellipsoidalis*), bur oak (*Q. macrocarpa*), eastern white pine and red pine (Cayford *et al.,* 1967). Where it occurs in mixtures it is often dominant, or codominant with trembling aspen, birch and sometimes red pine. Additional associated species on dry to mesic sites may include northern pin oak, bur oak, bigtooth aspen (*P. grandidentata*) and balsam poplar (*Populus balsamifera*) (Rudolph and Laidly, 1990). Less common associates include red maple (*Acer rubrum*), red oak (*Q. rubra*) and white oak (*Q. alba*) (Rudolf, 1958). In the east, it may be found infrequently with white oak, pin cherry (*Prunus pensylvanica*), gray birch (*B. populifolia*), red spruce (*P. rubens*) and pitch pine (*P. rigida*) (Rudolf, 1965; Greenwood *et al.,* 2002).

A variety of herbs and shrubs, ferns, mosses, and lichens are associated with jack pine, varying in distribution with soil type and moisture regime. Common on many site types across much of its extensive range are Virginia strawberry (*Fragaria virginiana*), late low-bush blueberry (*Vaccinium angustifolium*), common red bearberry (*Arctostaphylos uva-ursi*), short-awn mountain-rice (*Piptatherum pungens*), prickly rose (*Rosa acicularis*) and in the east sweetfern (*Comptonia peregrina*) (Cayford, 1963a, 1963b; Chrosciewicz, 1970, 1988b). On fresh to moist sites, the shrubs include alders (*Alnus viridis* subsp. *crispa* and *A. incana* subsp. *rugosa*), willows (*e.g.* gray willow [*Salix bebbiana*]), choke cherry (*P. virginiana*), pin cherry and beaked hazelnut (*Corylus cornuta*). Other common species on moist sites include Canadian bunchberry (*Cornus canadensis*), arctic sweet-coltsfoot (*Petasites frigidus* var. *palmatus*), bluejoint (*Calamagrostis canadensis*), rusty Labrador-tea (*Ledum groenlandicum*), American twinflower (*Linnaea borealis* subsp. *americana*) and common red raspberry (*Rubus idaeus* subsp. *strigosus*) (Chrosiewicz, 1983). Associated ferns may include northern bracken fern (*Pteridium aquilinum*). On drier sites, associates include reindeer-mosses (*e.g. Cladonia rangiferina*) and other lichens, Canadian small pussytoes (*Antennaria howellii* subsp. *canadensis*) and sometimes prairie redroot (*Ceanothus herbaceus*) (Chrosciewicz, 1983), while a shrub layer may be

lacking (Rudolf, 1958). Throughout much of its range, Schreber's moss (*Pleurozium schreberi*) is a common associate on many sites; other mosses and club-mosses may include juniper-leaf hair moss (*Polytrichum juniperinum*), glittering feather moss (*Hylocomium splendens*), ostrich-plume feather moss (*Hypnum crista-castrensis*) and stiff ground-pine (*Lycopodium annotinum*) (Rudolf, 1958; Cayford, 1963b; Chrosciewicz, 1970, 1988a, 1988b). In the eastern portion of its range, rhodora (*Rhododendron canadense*) is a common associate (Rudolf, 1965).

7.3. Competition, succession, and stand structure

Jack pine is a shade-intolerant, early-seral, relatively fast-growing and short-lived species. It is slightly more tolerant than aspen, birch and tamarack, but less so than many other conifers it is associated with such as black spruce, white spruce and balsam fir. Throughout much of its range, the major causes of catastrophic tree mortality and stand loss are fire in the west, and a mix of fire and budworm in the east. Jack pine stands are particularly susceptible: they commonly occur on drier sites, foliage is highly combustible and the bark thin. Indeed, the species may be adapted to facilitating fire spread (Kelsall *et al.,* 1977). Trees are often girdled and killed by fire (Cayford and McRae, 1983). In the central and northern portions of its range, jack pine succession is closely linked to forest fire regimes (Conkey *et al.,* 1995). With its serotinous cones, multi-cohort aerial seed banks, and early rapid growth, the species is well adapted to re-establishing sites following fire (Greene *et al.,* 2004). It can recolonise very large burns where it was present in the pre-fire landscape (Greene *et al.,* 1999). As most seedlings establish within 2 or 3 years of a fire, the result is often extensive even-aged stands. Greene and Johnson (1999) found that jack pine re-establishes roughly in proportion to its pre-fire basal area, reflecting in part the available seed bank. It may be found to occupy a broad range of site types after a burn, from muskeg (peatland) to ridge tops (Farrar, 1995; Pellerin and Lavoie, 2003).

Fire frequency is critical in determining jack pine population dynamics. Long-term maintenance of local populations depends on fire return intervals that are less than the average lifespan of individual trees (Desponts and Payette, 1992), but long enough for the development of adequate seed banks. A frequency of less than 15 to 20 years may result in local elimination, because of inadequate seed banks and the loss of potential future seed producers (Farrar, 1995; Greene and Johnson, 1999). Frequent fires in the Lake States may result in its elimination in favour of oaks such as northern pin oak (Rudolf, 1965). Conversely, at the limits of its range in northern Québec, jack pine is succeeded by black spruce in areas with long fire intervals (Desponts and Payette, 1992). The absence of the natural fire regime would result in elimination of jack pine from parts of its range, to be replaced by more shade-tolerant competitors (Cayford and McRae, 1983).

In the absence of fire in the Boreal and the Great Lakes–St. Lawrence Forest Regions, jack pine is often succeeded by the more tolerant black spruce, white spruce and balsam fir (Cayford *et al.,* 1967). On productive sandy sites in Minnesota, it may be succeed by red pine, then white pine and mixed hardwoods; in some cases succession is directly to hardwoods such as white birch and trembling aspen (Rudolf, 1965). In the Boundary Waters Canoe Area of northern Minnesota, succession may be to black spruce – feather moss forest types, or balsam fir – white birch – white spruce types (Cayford and McRae, 1983).

In some parts of its range jack pine is self-replacing even in the absence of fire, typically because of an ability to survive on sites too harsh for competitors. Desponts and Payette (1992) found in northern Québec that it was able to regenerate on sites of exposed mineral substrates. Among marginal disjunct coastal populations in Maine, Conkey *et al.,* (1995) observed continuous regeneration on sites too poor and shallow for other species; cone serotiny was low in these populations (negating the need for fire to achieve seed dispersal). In the southwestern part of its range, jack pine may continuously regenerate on very dry sites (Cayford *et al.,* 1967).

7.4. Species interactions and dynamics

A variety of insects affect the survival and growth of jack pine. Jack pine budworm (*Choristoneura pinus pinus*) is one of the most significant defoliators in central Canada and the Lake States (Howse, 1984). It feeds mainly on new growth, preferring male strobili clusters and new foliage, and can cause growth loss, top kill and mortality (Conway *et al.,* 1999). Swaine jack pine sawfly (*Neodiprion swainei*) is also an economically important pest, particularly in eastern Canada. Feeding on needles, it causes top kill, and if populations are sufficient, tree mortality occurs within 1 year or more typically 3 to 4 years (Howse, 1984).

In jack pine seed orchards in Wisconsin, the mirid *Platylygus luridus* has caused conelet abortion at rates of 51 to 87% (Rauf *et al.,* 1984). De Groot and Schnekenburger (1999) found that white pine weevil and eastern pine shoot borer respectively damaged 14.5% and 26.6%, of trees in an open-pollinated family test in Ontario. These two pests may significantly reduce height growth in the Lake States (Hodson *et al.,* 1982). Other damaging insects include root borers (*e.g.* pales weevil, *Hylobius pales*), shoot and stem borers (*e.g.* northern pine weevil, *Pissodes approximatus*), needle miners (*e.g. Argyrotaenia tabulana*) and root feeders (*Phyllophaga* spp.) (Rudolph and Laidly, 1990).

Armillaria root rot (*Armillaria mellea*) frequently kills seedlings and juvenile stands. Infection by sweetfern blister rust results in cankers that reduce the commercial value of trees; volume growth can be reduced by 20%, and younger trees killed (McGauley and Gross, 1984). *Scleroderris* canker occurs throughout the range of jack pine and often causes mortality of infected seedlings. Common foliar diseases include needle rust (*Coleosporium asterum*), needlecast and Diplodia tip blight (*Sphaeropsis sapines*).

A number of vertebrate species may damage or kill jack pine. Chrosciewicz (1988a) found that snowshoe hares (*Lepus americanus*) clipped tops of more than 40% of natural and seeded seedlings, although most damaged plants survived. In western Manitoba, seedlings may be damaged by elk (*Cervus canadensis*) when its population levels are high (Rudolph and Laidly, 1990). Jack pine is generally considered a food of medium preference for deer (*Odocoileus*) (Cayford and McRae, 1983). Red squirrels (*Tamiasciurus hudsonicus*) frequently harvest jack pine cones; Rauf *et al.,* (1985) reported losses of 10% of conelets and 30% of cones.

Essential to Kirtland's warbler (*Dendroica kirtlandii*), pure young jack pine stands in north-central Michigan provide the only nesting habitat used by this endangered bird (Benzie, 1977; Farrar, 1995).

8. Reforestation practices

8.1. Provenance transfer

Where the jack pine range overlaps that of lodgepole pine in the west, the latter is preferred for commercial purposes. Extensive jack pine provenance testing has been carried out in eastern Canada and the Lake States. In numerous studies, local seed sources have typically performed well, but provenance transfer may further enhance performance.

The best sources within Saskatchewan were from the province's west and south (Klein, 1989a). Jack pine rangewide provenance testing in Manitoba found that better sources were from western and central Ontario and Québec, Minnesota and Manitoba, whereas poorly performing provenances included populations from eastern Ontario and Québec, northeastern USA, the Maritimes, and Michigan, Saskatchewan, Alberta and the Northwest Territories (Klein, 1990). For eastern Canada, Magnussen and Yeatman (1988b) recommended the use of well-defined breeding zones, and larger breeding zones in southern, milder areas. Matyas and Yeatman (1992) developed an ecological distance index based upon heat sum units and latitude for recommending transfer distances in Ontario. Using this index, a moderate northward transfer was recommended.

In the Great Lakes region including Ontario and the Lake States (Michigan, Wisconsin and Minnesota), for best growth Morgenstern and Teich (1969) recommended moving northern provenances 2-3° southward, and southern provenances 1-2° northward. Within this region, northern provenances generally grew better when transferred southward, but best growth on southern sites was from southern provenances (Yeatman, 1974). Local sources often were among the best performers, except for slow-growing populations from east of Lake Nipigon and north of Lake Superior in Ontario (Yeatman and Morgenstern, 1979).

Rudolf and Yeatman (1982) summarised various early studies. Schnare (1969) found that southern provenances grew taller in Missouri. In a Nebraska test, best sources were from Ontario and Québec, and shortest trees from the Northwest Territories (Sprackling and Read, 1975). Sources from Lower Michigan exhibited the best performance in Michigan, Wisconsin, Minnesota and Petawawa, Ontario (King, 1966; Alm and Jensen, 1969; Canavera and Wright, 1973; Yeatman, 1974). Local sources were also superior (Schantz-Hansen and Jensen, 1952; King, 1966; Jeffers and Jensen, 1980; Rudolph and Yeatman, 1982). Rudolph and Yeatman (1982) recommended that movement of sources up to 160 km northward could increase growth in the Lake States region. Jeffers (1971) had recommended using only local seed in Lower Michigan and in Minnesota, and using a mixture of local and Lower Michigan seed in Wisconsin and Upper Michigan.

Hyun (1979) used cluster analysis to analyze rangewide populations tested in Minnesota. He obtained five clusters, with the Lake States cluster and the Northwest Territories–Alberta–northern Québec cluster being the most distinct. Jeffers and Jensen (1980) assembled populations tested at fourteen Lake States sites into three groupings. The northernmost group was from the area with the harshest climates, and these sources had the worst performance and highest eastern gall rust incidence. The southernmost group, with the mildest climate, had best performance and lowest eastern gall rust incidence.

Six provenances from Ontario were tested in New Zealand at two sites (one on the North Island, one on the South Island) (Sweet and Thulin, 1963). The tallest and earliest to flower sources were the southernmost from the mildest climates.

8.2. Breeding programs

Jack pine breeding programs have been established throughout much of its natural range. Tree improvement programs are particularly important in the Lake States and central and eastern Canada. Jack pine is considered a minor timber species in Nova Scotia and Prince Edward Island, but the second most important in New Brunswick, which has completed establishment of second generation programs (Tosh and McInnis, 2000). Ontario is establishing second generation progeny tests (Boysen *et al.,* 2000), while Manitoba was planning to establish third generation tests (Klein, 1998). Lodgepole pine is of greater interest than jack pine within Alberta. However, Alberta has made both jack pine and jack pine × lodgepole pine hybrid wild stand selections and established a clonal jack pine seed orchard for the northeastern boreal region, and is testing wood density and fibre length of superior parent trees (Hansen *et al.,* 1997). Minnesota and Michigan have established second generation seed orchards (Rudolph 1984; Stine *et al.,* 1995), and seed orchards are also in place in Wisconsin and Maine (Rudolph 1984; Carter and Simpson, 1985).

Selection is primarily based upon growth and stem quality traits; pest resistance may also be important. Commonly selection is based upon age-10 measurements, although New Brunswick makes selections on height at age 7 (Fowler, 1986), and selections are made in Manitoba and Saskatchewan tests based on measurements at age 20 (Klein, 1998). First generation programs are usually based on open-pollinated family selection. Sometimes, as in Minnesota, progeny tests are rogued for conversion to seed orchards after measuring (Stine *et al.,* 1995). Second generation breeding strategies are more diverse, and typically include stratified breeding populations. New Brunswick uses a combination of polycrossing for breeding value estimation and single-pair assortative mating to produce full-sib crosses,

which are assigned to sublines (Fowler, 1986). Saskatchewan (Sande and Corriveau, 2000), Manitoba (Klein, 1998) and the Lake States (Rudolph 1984) are also using sublined breeding populations. Ontario is using a nucleus breeding system, with the breeding population substructured into an elite population produced by single-pair mating and an open-pollinated infusion population (Joyce and Nitschke, 1993).

Provenance tests generally provide information on which the breeding zone delineation and program establishment are based. Ontario has numerous jack pine breeding zones, but has amalgamated a number of them in the northwestern part of the province based on Focal Point Seed Zone methodology (Parker and van Niejenhuis, 1996). New Brunswick comprises only one breeding zone (Fowler, 1986); there are three in Manitoba and Saskatchewan (Klein, 1982).

New Brunswick is using accelerated growth cycles at the greenhouse stage to rear grafts prior to assigning them to sublines, and is experimenting with miniaturised jack pine meadow seed orchards, which have been established using accelerated-grown stock (Simpson, 1997). Supplemental mass pollination using the best clones as pollen sources is used within the meadow orchards to increase amounts of filled seed (Simpson, 1997). Realised gain trials have also been established (Simpson and Tosch, 1997).

8.3. Reproductive propagation

8.3.1. Flower induction

Jack pine is one of the youngest pines to flower, and under natural conditions may produce male and female strobili at 3 years of age (Righter, 1939), albeit at low frequencies (Rudolph 1966b). Johnson and Critchfield (1978) found female strobili on 10-month-old seedlings of a precocious lodgepole pine × jack pine hybrid cross. More typically, jack pine begins to reproduce at 5 to 10 years when open-grown, and 10 to 25 years in closed stands (Roe, 1963). Production of first male strobili usually lags 2 to 3 years behind that of female strobili, and may contribute to poor seed yield (Cecich, 1983a).

Strobili production can be enhanced using cultural practices. Rudolph (1966b) applied an accelerated-growth regime that included a 20-hour photoperiod in a greenhouse environment prior to transplanting seedlings to a nursery bed. Up to 62% of 23-month-old seedlings produced strobili, averaging 2 per tree, of which 51% developed into mature cones yielding an average of 26 seeds per cone and 77% germination. Rudolph (1979a) induced female strobili on 1% of 12-month-old seedlings that had been given 10 weeks of favourable greenhouse conditions prior to transplanting. Male strobili production did not respond as readily to accelerated-growth treatments (Rudolph 1966b, 1979a). Cecich and Bauer (1987) used artificial photoperiod and temperature regimes to apply a compressed reproductive cycle to 2-year-old seedlings, which produced seed 9 months after the pollination of female strobili (rather than the natural 16 months), although the yield of filled seeds was low. Moisture stress applied as three drying cycles from June to August yielded four times more female strobili on 12-month-old accelerated-growth seedlings than controls (Riemenschneider, 1985). In contrast, Fogal *et al.,* (1995) found that moisture stress slightly reduced female strobilus production. Nitrogen deficiency enhanced pollen strobilus production on 2-, 3- and 6-year-old seedlings (Fogal *et al.,* 1994, 1995).

Gibberellin, in particular $GA_{4/7}$, has been used to promote early and enhanced male and female strobilus production. Biweekly foliar spray applications at concentrations of 400 and 600 mg per L, timed to affect reproductive bud differentiation, have been successful (Cecich, 1983b; Fogal *et al.,* 1994; Ho and Hak, 1994). Fogal *et al.,* (1996) were able to increase production of pollen strobili but not seed strobili using stem injections of $GA_{4/7}$. The effects of accelerated-growth treatment may be enhanced by the application of $GA_{4/7}$ (Cecich, 1981, 1983a; Cecich *et al.,* 1994). Gibberellin may help to compensate for variation in flowering among families (Cecich *et al.,* 1994); treatment periods may be timed to capture family differences in phenology (Ho and Hak, 1994).

8.3.2. Vegetative propagation

Long-shoot cuttings from juvenile jack pine generally root well, with rooting frequencies of 70 to 95% reported (Armson *et al.,* 1975; Browne *et al.,* 1996, 1997a). However, attempts to root cuttings from donors greater than 5 or 6 years old have met with less success. Zsuffa (1974) achieved an average of 6.6% rooting from 6- and 10-year-old donors; Potapova (1998) got no rooting from donors 20 to 25 years old. Browne *et al.,* (1997a) were able to attain 80% rooting using 3-year-old donors, but only 20% (with same treatment) on cuttings from 7-year-old donors. They identified a transitional period at 4 to 6 years of age – between juvenility and maturity – within which rooting ability drops significantly. In addition to rooting ability, the length and number of roots produced by cuttings also show an age-related decline (Browne *et al.,* 1997a, 1997b). The change in rooting ability of juvenile and mature cuttings has been linked to differences in net photosynthetic rate, carbohydrate partitioning, and starch metabolism (Haissig, 1989). Decreases in rooting ability with increasing donor stock age are well documented in pines (*e.g.* Girouard, 1971).

Several studies have examined the potential of rooting proliferated dwarf shoots, which are needle fascicles in which the diminutive shoot apex develops into a functional long shoot (Ewers, 1983), a response often induced by pruning. Rudolph and Nienstaedt (1964) rooted needle fascicles; only those from pruned donors showed fascicular bud development, rooted well and produced normal stems. Browne *et al* (1997b) achieved 87%, 86%, 60% and 49% rooting, using 2-, 4-, 6- and 8-year-old proliferated dwarf shoots respectively, but achieved only 20 to 24% rooting of 11-year-old shoots. Browne *et al.,* (2001) significantly increased the production of proliferated dwarf shoots on pruned seedlings with foliar applications of benzyladenine (BA), although maximum rates had deleterious effects on subsequent rooting; the optimum treatment yielded 22 to 29 rooted cuttings per 6-month-old donor. Rooting of proliferated dwarf shoots may thus provide an economic means of expanding seed of superior controlled cross pollinations into production populations (Klein *et al.,* 1995).

Treatment of cuttings with growth hormones may increase rooting frequency. Naphthalene acetic acid (NAA) increases speed and abundance of adventitious root primordium development (Haissig, 1982). NAA can significantly increase rooting (Browne *et al.,* 2000), and it may delay (but not halt) the age-related decline in rooting ability (Browne *et al.,* 1997a). Synthetic auxins (IBA, NP-IBA and P-ITB) improved rooting of cuttings from young seedlings (Haissig, 1983, 1990). Rooting ability may be affected by length of cutting (Zsuffa, 1974), shoot type (dwarf shoot vs. long shoot) (Browne *et al.,* 1997b), and crown position of the cutting (Browne *et al.,* 1996, 1997a). Treatment of donor stock with chilling and moderate fertiliser levels enhanced rooting ability (Browne *et al.,* 2000), whereas shade treatment was deleterious (Haissig, 1990).

Significant genetic variation in rooting ability of jack pine exists (Zsuffa, 1974). Exceptional individuals have been observed; for example, Browne *et al.,* (1997a) noted that two 30-year-old ortets yielded 57 to 58% rooting. Although the potential for selecting for good rooting clones exists, genetic correlations between rooting and other traits of economic importance (*e.g.* volume growth) have not been reported.

The first successful attempts at using somatic embryogenesis to propagate jack pine were carried out in 1995, but jack pine is recalcitrant to *in vitro* culture (*e.g.* Briand *et al.,* 1998; Park *et al.,* 2000; Pelletier and Laliberté. 2000). Charbonneau and Laliberté (2004) achieved promising results by culturing meristematic nodules from zygotic embryos, though organogenesis has yet to be optimised.

8.4. Stock deployment

The deployment methods for jack pine vary across jurisdictions, but typically consist of a combination of manual or machine planting of bare root, transplant or container stock, and aerial or spot seeding. The principal method in New Brunswick and Nova Scotia has been manual planting of

2-0 bare root and container stock; seeding is rare (Barteaux and Bailey, 1986). Machine and hand planting of 3-0 and 2-0 stock have been used in Saskatchewan, container stock less so (Little, 1984). In Québec, planting of 3-0 and 2-0, as well as 2-1 and 1.5-1.5 transplants and container stock have been practiced, and aerial seeding and spot ground seeding used (Couture and Dancause, 1984). In Ontario, planting of bare root and container stock and aerial seeding are practiced.

8.5. Conservation of genetic resources

Jack pine is a widespread, predominantly outcrossing species with high levels of gene flow *via* pollen dispersal. Most of the genetic variation is found within populations. Differences in diversity measures between natural stands and plantations have not been observed (Knowles, 1985). However, resource exploitation, habitat fragmentation and land-use change may contribute to depletion of the genetic resource. Populations in marginal habitats, such as at the limits of its range, or those isolated and undergoing more frequent inbreeding, are most vulnerable to loss in genetic diversity. Populations with low levels of diversity may be unable to adapt to changing environmental conditions such as climate change due to global warming, which would be expected to exert strong selective pressures. Selection against genotypes may occur during cone collection, nursery seedling culture, tree improvement programs, precommercial thinning, and harvesting.

In situ conservation may include maintenance of forest reserves, protected areas, and migration corridors. Reserves sufficient to allow for natural evolutionary processes to occur such as gene flow, mating, selection, and mutation are preferred. Seed transfer and stand management practices that are managed with the goal of increasing diversity may provide other conservation measures. Populations most at risk of genetic depletion need to be identified, and steps taken to protect them. Baseline biological data and knowledge regarding population structure, such as the information from provenance studies, are needed when developing effective conservation strategies. Fowler (1986) described a reserve-stand policy for *in situ* conservation of jack pine populations in New Brunswick. Boyle (1992), in a summary of forest genetic conservation activities in Canada, listed two limestone ecotype conservation areas in Ontario that include jack pine, and reserve stands of jack pine in Québec, Ontario and Manitoba.

Ex situ conservation offers additional protection of the gene pool, and may be necessary for populations most at risk. *Ex situ* reserves include seed bank storage, germplasm cryopreservation, clone banks, arboreta, seed orchards, field trials, and plantations. Boyle (1992) listed various ongoing *ex situ* activities in Canada. Most of the Canadian provinces maintain seed banks. The National Forest Genetic Resources Centre, which includes the National Tree Seed Centre, is operated by Natural Resources Canada at the Atlantic Forestry Centre with the goal of gene conservation of native species (Simpson and Daigle, 1998). Rangewide seed collections of jack pine are being obtained. Active gene management that could be incorporated into breeding programs includes strategies such as the multiple population breeding system, which is designed to manage populations in breeding programs in such a way that genetic diversity is maintained or even increased despite reduction of the population size through ongoing selection (Eriksson *et al.,* 1993).

9. Summary

Jack pine is one of the most widespread tree species and is the most widespread pine species in North America, growing farther north than any other pine. It is of significant economic importance in Canada and in the Lake States of the USA, where it is harvested for pulp and lumber products.

The population dynamics of jack pine are closely linked to fire regimes, to which the species is well adapted. With serotinous cones, large aerial seed banks and rapid early growth, it readily re-establishes following burns. In the absence of fire, jack pine is typically succeeded by more tolerant species; however, on some poor sites in marginal portions of its range it can regenerate without fire. Although its best growth is achieved on fertile sites of fresh to moist sands and loams, jack pine can grow on very dry, shallow and infertile sites on which competing tree species cannot survive.

Jack pine is precocious and responds well to flower induction treatments, making it suitable for accelerated breeding programs. It does not root vegetatively in nature, nor respond as readily as some other conifers to rooting treatments. Although vegetative propagation may be useful for expanding specialised crosses, it currently is not practical for general stock production.

Jack pine exhibits genetic variation within and among populations in many traits, including height and diameter growth, wood quality, cone and seed characteristics, and pest resistance. Breeding programs exist throughout much of its range, and are of particular importance in the Lake States and central and eastern Canada. Selection is based primarily upon growth and stem traits, as well as pest resistance.

References

Adams, G.W., and E.K. Morgenstern. 1991. Multiple-trait selection in jack pine. Can. J. For. Res. 21: 439-445.

Ahlgren, C.E. 1959. Some effects of fire on forest reproduction in northeastern Minnesota. J. For. 57: 194-202.

Ahlgren, C.E. 1974. Effects of fires on temperate forests: North Central United States. Pp. 195-223 *in* T.T. Kozlowski and C.E. Ahlgren (eds.), Fire and ecosystems. Academic Press, New York.

Alm, A.A., and R.A. Jensen. 1969. Results of a Lake States jack pine seed source test in east-central Minnesota. Minn. For. Notes 200. 4 pp.

Almendinger, J.C. 1992. The late Holocene history of prairie, brush-prairie, and jack pine (*Pinus banksiana*) forest on outwash plains, north-central Minnesota, USA. Holocene 2: 37-50.

Anderson, N.A., and R.L. Anderson. 1965. The susceptibility of jack pine and lodgepole pine and their hybrids to sweetfern rust and eastern gall rust. USDA Forest Service Lake States Forest Experiment Station Research Note LS-56. 4 pp.

Arend, J.L., N.F. Smith, S.H. Spurr and J.W. Wright. 1961. Jack pine geographic variation – Five-year results from Lower Michigan tests. Papers of the Michigan Academy of Science, Arts and Letters 46: 219-237.

Armson, K.A., J. Pérez de la Garza and R.J. Fessenden. 1975. Rooting cuttings of conifer seedlings. For. Chron. 51: 109-110.

Arseneault, D., and L. Sirois. 2004. The millennial dynamics of a boreal forest stand from buried trees. J. Ecol. 92: 490–504.

Asselin, H., S. Payette, M.-J. Fortin and S. Vallée. 2003. The northern limit of *Pinus banksiana* Lamb. in Canada: Explaining the difference between the eastern and western distributions. J. Biogeogr. 30: 1709–1718.

Barteaux, M.K., and R.E. Bailey. 1984. Status report on the management of jack pine in the Provinces of New Brunswick and Nova Scotia. Pp. 14-19 *in* Proceedings of Jack Pine Symposium. Canadian Forest Service, Great Lakes Forest Research Centre, COFJRC Symposium Proceedings O-P-12.

Batzer, H.O. 1961. Jack pine from Lake States seed sources differ in susceptibility to attack by the white-pine weevil. USDA Forest Service Lake States Forest Experiment Station Technical Note No. 595. 2 pp.

Batzer, H.O. 1962. White-pine weevil damage differs significantly by seed source on two northern Minnesota jack pine plantations. USDA Forest Service Lake States Forest Experiment Station Technical Note No. 618. 2 pp.

Benzie, J.W. 1977. Manager's handbook for jack pine in the North Central States. USDA Forest Service North Central Forest Experiment Station General Technical Report NC-32. 18 pp.

Blake, T.J., and C.W. Yeatman. 1989. Water relations, gas exchange, and early growth rates of outcrossed and selfed *Pinus banksiana* families. Can. J. Bot. 67: 1618-1623.

Bolstad, S.B., H. Kang, R.P. Guries and T.L. Marty. 1991. Performance of inter- and intraprovenance crosses of jack pine in central Wisconsin. Silvae Genet. 40: 124-130.

Boyle, T.J.B. 1992. Forest tree genetic conservation activities in Canada. Forestry Canada Information Report ST-X-4. 50 pp.

Boysen, B., P. Charrette, R. Ford, D. Joyce and C. Nielsen. 2000. Ontario's genetic resource management program. Pp. 78-80 *in* Proceedings of the 27th Meeting of the Canadian Tree Improvement Association, Sault Ste. Marie, Ontario, Part 1.

Briand, C.H., S. Gauthier, J.-F. Lemay, S. Laliberte and M.-F. Tremblay. 1998. Relationship between field performance, family, embryo morphology, and isozyme heterozygosity, and *in vitro* reactivity in jack pine. Can. J. For. Res. 28: 98-105.

Browne, R.D., C.G. Davidson and S.M. Gobin. 1996. Effects of crown position and plant age on rooting of jack pine long-shoot cuttings. Tree Planters' Notes 47: 100-104.

Browne, R.D., C.G. Davidson and S.M. Enns. 2000. Improvements in asexual multiplication procedures for jack pine (*Pinus banksiana*). New For. 19: 259-278.

Browne, R.D., C.G. Davidson, T.A. Steeves and D.I. Dunstan. 1997a. Effects of ortet age on adventitious rooting of jack pine (*Pinus banksiana*) long-shoot cuttings. Can. J. For. Res. 27: 91-96.

Browne, R.D., C.G. Davidson, T.A. Steeves and D.I. Dunstan. 1997b. Rooting proliferated dwarf shoot cuttings of jack pine (*Pinus banksiana*). Can. J. For. Res. 27: 97-101.

Browne, R.D., C.G. Davidson, S.M. Enns, T.A. Steeves and D.I. Dunstan. 2001. Foliar application of benzyladenine (BA) as a means to enhance shoot production for asexual propagation of young jack pine (*Pinus banksiana* Lamb.) seedlings. New For. 22: 229-237.

Burdon, R.D. 2002. An introduction to pines. Pp. x-xxi *in* CABI Forestry Compendium (ed.), Pines of silvicultural importance. CABI Publishing, Wallingford, U.K.

Canavera, D.S. 1975. Variation among the offspring of selected Lower Michigan jack pines. Silv Genet. 24: 12-15.

Canavera, D.S., and J.W. Wright. 1973. A 4-year provenance test of jack pine. Michigan State University Agricultural Experiment Station Research Paper 204. 7 pp.

Cantin, D., M.F. Tremblay, M.J. Lechowicz and C. Potvin. 1997. Effects of CO_2 enrichment, elevated temperature, and nitrogen availability on the growth and gas exchange of different families of jack pine seedlings. Can. J. For. Res. 27: 510-520.

Carter, K.K., and J.D. Simpson. 1985. Status and outlook for tree improvement programs in the Northeast. Northern J. Appl. For. 2: 127-131.

Carter, K.K., G.W. Adams, M.S. Greenwood and P. Nitschke. 1990. Early family selection in jack pine. Can. J. For. Res. 20: 285-290.

Cayford, J.H. 1959. Germination and survival of jack pine and red pine after scarification in southeastern Manitoba. Canada Department of Northern Affairs and Nat. Resources, Forestry Branch, Technical Note No. 78. 14 pp.

Cayford, J.H. 1963a. Reproduction and residual stand development following cutting in red pine – jack pine stands in Minnesota. Canada Department of Forestry, Forest Research Branch, Publication No. 1010. 19 pp.

Cayford, J.H. 1963b. Some factors influencing jack pine regeneration after fire in southeastern Manitoba. Canada Department of Forestry, Forest Research Branch, Publication No. 1016. 16 pp.

Cayford, J.H., and D.J. McRae. 1983. The ecological role of fire in jack pine forests. Pp. 183-199 *in* R.W. Wein and D.A. MacLean (eds.), The role of fire in northern circumpolar ecosystems. John Wiley & Sons Ltd., Chichester, England.

Cayford, J.H., Z. Chrosciewicz and H.P. Sims. 1967. A review of silvicultural research in jack pine. Canada Department of Forestry and Rural Development, Forestry Branch; Department Publication No. 1173.

Cecich, R.A. 1981. Applied gibberellin $A_{4/7}$ increases ovulate strobili production in accelerated growth jack pine seedlings. Can. J. For. Res. 11: 580-585.

Cecich, R.A. 1983a. Effect of weekly and bi-weekly spraying of $GA_{4/7}$ on flowering in jack pine seedlings. Pp. 292-297 *in* Proceedings of the 7th North American Forest Biology Workshop, Lexington, Kentucky, July 26-28, 1982.

Cecich, R.A. 1983b. Flowering in a jack pine seedling seed orchard increased by spraying with gibberellin $A_{4/7}$. Can. J. For. Res. 13: 1056-1062.

Cecich, R.A., and E.O. Bauer. 1987. The acceleration of jack pine seed development. Can. J. For. Res. 17: 1408-1415.

Cecich, R.A., H. Kang and W. Chalupka. 1994. Regulation of early flowering in *Pinus banksiana*. Tree Physiol. 14: 275-284.

Charbonneau J. and S. Laliberté. 2004. *In vitro* and cellular responses on jack pine embryos to three imbibition parameters. *In vitro* cellular & developmental biology. Plant. 40: 559-566.

Cheliak, W.M., B.P. Dancik, K. Morgan, F.C.H. Yeh, and C. Strobeck. 1985. Temporal variation of the mating system in a natural population of jack pine. Genetics 109: 569-584.

Chrosciewicz, Z. 1963. The effects of site on jack pine growth in northern Ontario. Canada Department of Forestry, Forest Research Branch, Publication No. 1015. 28 pp.

Chrosciewicz, Z. 1970. Regeneration of jack pine by burning and seeding treatments on clear-cut sites in central Ontario. Canada Department of Fisheries and Forestry, Canadian Forestry (Forest???) Service, Ontario Region Information Report O-X-138. 13 pp.

Chrosciewicz, Z. 1983. Jack pine regeneration following postcut burning and seeding in southeastern Manitoba. Canadian Forest Service, Northern Forest Research Centre, Information Report NOR-X-252. 10 pp.

Chrosciewicz, Z. 1988a. Forest regeneration on burned, planted, and seeded clear-cuts in central Saskatchewan. Canadian Forest Service, Northern Forest Research Centre, Edmonton, Alberta; Information Report NOR-X-293. 16 pp.

Chrosciewicz, Z. 1988b. Jack pine regeneration following postcut burning under seed trees in central Saskatchewan. For. Chron. 64: 315-319.

Conkey, L.E., M. Keifer and A. Lloyd. 1995. Disjunct jack pine (*Pinus banksiana* Lamb.) structure and dynamics, Acadia National Park, Maine. Écoscience 2: 168-176.

Conway, B.E., D.G. McCullough and L.A. Leefers. 1999. Long-term effects of jack pine budworm outbreaks on the growth of jack pine trees in Michigan. Can. J. For. Res. 29: 1510-1517.

Couture, G., and A. Dancause. 1984. Status report on the management of jack pine in the Province of Quebec. Pp. 29-37 *in* Proceedings of Jack Pine Symposium. Canadian Forest Service, Great Lakes Forest Research Centre, COFJRC Symposium Proceedings O-P-12.

Critchfield, W.B. 1975. Interspecific hybridisation in *Pinus*: A summary review. Pp. 99-105 *in* D.P. Fowler and C.W. Yeatman, Proceedings of the 14th Meeting of the Canadian Tree Improvement Association, Part 2. Fredericton, New Brunswick.

Critchfield, W.B. 1980. Genetics of lodgepole pine. USDA Forest Service Research Paper WO-37, Washington, D.C. 57 pp.

Critchfield, W.B. 1984. Impact of the Pleistocene on the genetic structure of North American conifers. Pp. 70-114 *in* R.M.. Lanner (ed.), Proceedings of the 8th North American Forest Biology Workshop, Utah.

Critchfield, W.B. 1985. The late Quaternary history of lodgepole and jack pines. Can. J. For. Res. 15: 749-772.

Curtis, J.D., and R.A. Popham. 1972. The development anatomy of long-branch terminal buds of *Pinus banksiana*. Am. J. Bot. 59: 194-202.

Dancik, B.P., and F.C.H. Yeh. 1983. Allozyme variability and evolution of lodgepole pine (*Pinus contorta* var. *latifolia*) and jack pine (*Pinus banksiana*) in Alberta. Can. J. Genet. Cytol. 25: 57-64.

Danzmann, R.G., and G.P. Buchert. 1983. Isozyme variability in central Ontario jack pine. Pp. 232-248 *in* R.T. Eckert (ed.), Proceedings of the 28th Northeastern Forest Tree Improvement Conference, Durham, New Hampshire, 1982.

Davis, M.B. 1976. Pleistocene biogeography of temperate deciduous forests. Geosci. Man 13: 13-26.

de Groot, P., and F. Schnekenburger. 1996. Cone traits of jack pine and black spruce in young seedling seed orchards. New For. 12: 279-291.

de Groot, P., and F. Schnekenburger. 1999. Variation in damage by terminal shoot insects among jack pine families. For. Ecol. Manag. 121: 251-255.

de Groot, W.J., P.M. Bothwell, S.W. Taylor, B.M. Wotton, B.J. Stocks and M.E. Alexander. 2004. Jack pine regeneration and crown fires. Can. J. For. Res. 34: 1634-1641.

Despland, E., and G. Houle. 1997. Climate influences on growth and reproduction of *Pinus banksiana* (Pinaceae) at the limit of the species distribution in eastern North America. Am. J. Bot. 84: 928-937.

Desponts, M., and S. Payette. 1992. Recent dynamics of jack pine and its northern distribution limit in northern Quebec. Can. J. Bot. 70: 1157-1167.

Desponts, M., and S. Payette. 1993. The Holocene dynamics of jack pine at its northern range limit in Québec. J. Ecol. 81: 719-727.

Di Giovanni, F., P.G. Kevan and M.E. Nasr. 1995. The variability in settling velocities of some pollen and spores. Grana 34: 39-44.

Di Giovanni, F., P.G. Kevan and J. Arnold. 1996. Lower planetary boundary layer profiles of atmospheric conifer pollen above a seed orchard in northern Ontario, Canada. For. Ecol. Manag. 83: 87-97.

Dong, J., and D.B. Wagner. 1993. Taxonomic and population differentiation of mitochondrial diversity in *Pinus banksiana* and *Pinus contorta*. Theor. Appl. Genet. 86: 573-578.

Dong, J., and D.B. Wagner. 1994. Paternally inherited chloroplast polymorphism in *Pinus*: Estimation of diversity and population subdivision, and tests of disequilibrium with a maternally inherited mitochondrial polymorphism. Genetics 36: 1187-1194.

Durzan, D.J., and V. Chalupa. 1968. Free sugars, amino acids, and soluble proteins in the embryo and female gametophyte of jack pine as related to climate at the seed source. Can. J. Bot. 46: 417-428.

Elias, T.S. 1980. The complete trees of North America: Field guide and natural history. Van Nostrand Reinhold, New York. 948 pp.

Eriksson, G., G. Namkoong and J.H. Roberds. 1993. Dynamic gene conservation for uncertain futures. For. Ecol. Manag. 62: 15-37.

Ernst, S.G., G. Howe, J.W. Hanover and D.E. Keathley. 1983. Genetic variation and gains of specific gravity and woody biomass in a jack pine half-sib progeny test in Michigan. Pp. 111-122 *in* Proceedings of the 3rd North Central Tree Improvement Conference, Wooster, Ohio.

Ewers, F.W. 1983. The determinate and indeterminate dwarf shoots of *Pinus longaeva* (bristlecone pine). Can. J. Bot. 61: 2280-2290.

Eyre, F.H., and R.K. LeBarron. 1944. Management of jack pine stands in the Lake States. USDA Technical Bulletin No. 863. 66 pp.

Farrar, J.L. 1995. Trees in Canada. Fitzhenry & Whiteside Ltd. / Natural Resources Canada, Canadian Forest Service, Markham / Ottawa, Ontario. 502 pp.

Fogal, W.H., S.M. Lopushanski, S.J. Coleman, H.O. Schooley and M.S. Wolynetz. 1995. Sexual expression in container-grown jack pine seedlings. Tree Physiol. 15: 439-442.

Fogal, W.H., G. Jobin, H.O. Schooley, S.J. Coleman and M.S. Wolynetz. 1996. Stem incorporation of gibberellins to promote sexual development of white spruce, Norway spruce, and jack pine. Can. J. For. Res. 26: 186-195.

Fogal, W.H., S.J. Coleman, M.S. Wolynetz, H.O. Schooley, S.M. Lopushanski and M.L. Anderson. 1994. Modifying sexual expression of containerized jack pine trees by topping, altering nitrogen and water supply and gibberellins. Can. J. For. Res. 24: 869-877.

Fowler, D.P. 1965a. Effects of inbreeding in red pine, *Pinus resinosa* Ait. IV. Comparison with other northeastern *Pinus* species. Silvae Genet. 14: 76-81.

Fowler, D.P. 1965b. Natural self-fertilisation in three jack pines and its implications in seed orchard management. For. Sci. 11: 55-58.

Fowler, D.P. 1986. Strategies for the genetic improvement of important tree species in the Maritimes. Canadian Forest Service, Maritimes Information Report M-X-156. 30 pp.

Fu, Y.B., P. Knowles and D.J. Perry. 1992. Pollen pool heterogeneity in jack pine (*Pinus banksiana* Lamb.): A problem for estimating outcrossing rates? Theor. Appl. Genet. 83: 500-508.

Fuller, J.L. 1997. Holocene forest dynamics in southern Ontario, Canada: Fine-resolution pollen data. Can. J. Bot. 75: 1714-1727.

Galloway, R. 1986. Jack Pine Working Group. Ontario Ministry of Natural Resources, Forest Resource Group, Silvicultural Guide Series. 40 pp.

Garrett, P.W. 1979. Species hybridisation in the genus *Pinus*. USDA Forest Service Northeast Forest Experiment Station Research Paper NE-436. 17 pp.

Gauthier, S., J.-P. Simon and Y. Bergeron. 1992. Genetic structure and variability in jack pine populations: Effects of insularity. Can. J. For. Res. 22: 1958-1965.

Giertych, M.M., and J.L. Farrar. 1962. A provenance study of jack pine seedlings. Silvae Genet. 11: 111-114.

Girouard, R.M. 1971. Vegetative propagation of pines by means of needle fascicles – A literature review. Canadian Forest Service, Centre de Recherche Forestière des Laurentides, Québec Region, Québec. Information Report Q-X-23. 16 pp.

Godbout, J., J.P. Jaramillo-Correa, J. Beaulieu, and J. Bousquet. 2005. A mitochondrial DNA minisatellite reveals the postglacial history of jack pine (*Pinus banksiana* Lamb.), a broad range North American conifer. Mol. Ecol. (*In press*).

Godt, M.J.W., J.L. Hamrick, M.A. Edwards-Burke and J.H. Williams. 2001. Comparisons of genetic diversity in white spruce (*Picea glauca*) and jack pine (*Pinus barksiana*) seed orchards with natural populations. Can. J. For. Res. 31: 943-949.

Govindaraju, D.R., D.B. Wagner, G.P. Smith and B.P. Dancik. 1988. Chloroplast DNA variation within individual trees of a *Pinus banksiana – Pinus contorta* sympatric region.Can. J. For. Res. 18: 1347-1350.

Greene, D.F., and E.A. Johnson. 1999. Recruitment of *Populus tremuloides*, *Pinus banksiana*, and *Picea mariana* into burns in the mixedwood boreal forest of central Saskatchewan. Can. J. For. Res. 29: 462-473.

Greene, D.F., J. Noël, Y. Bergeron, M. Rousseau and S. Gauthier. 2004. Recruitment of *Picea mariana*, *Pinus banksiana*, and *Populus tremuloides* across a burn severity gradient following wildfire in the southern boreal forest of Quebec. Can. J. For. Res. 34: 1845-1857.

Greene, D.F., J.C. Zasada, L. Sirois, D. Kneeshaw, H. Morin, I. Charron and M.-J. Simard. 1999. A review of the regeneration dynamics of North American boreal forest tree species. Can. J. For. Res. 29: 824-839.

Greenwood, M.S., W.H. Livingston, M.E. Day, S.C. Kenaley, A.S. White and J.C. Brissette. 2002. Contrasting modes of survival by jack and pitch pine at a common range limit. Can. J. For. Res. 32: 1662–1674.

Haissig, B.E. 1982. Carbohydrate and amino acid concentrations during adventitious root primordia development in *Pinus banksiana* Lamb. cuttings. For. Sci. 28: 813-821.

Haissig, B.E. 1983. N-phenyl indolyl-3-butyramide and phenyl indole-3-thiolobutyrate enhance adventitious root primordium development. Physiol. Plant. 57: 435-440.

Haissig, B.E. 1989. Carbohydrate relations during propagation of cuttings from sexually mature *Pinus banksiana* trees. Tree Physiol. 5: 319-328.

Haissig, B.E. 1990. Reduced irradiance and applied auxin influence carbohydrate relations in *Pinus banksiana* cuttings during propagation. Physiol. Plant. 78: 455-461.

Hansen, C.R., N.K. Dhir, L. Barnhardt, R.-C. Yang, K. Yakimchuk, J. Quinn, S. Hoberg, M.A. Mochulski and C. Andriuk. 1997. Genetics and tree improvement program, 1995-97, Alberta Land and Forest Service. Pp. 126-130 *in* Proceedings of the 26th Meeting of the Can. Tree Improvement Association, Ste. Foy, Québec, Part 1.

Hay, I., D. Lachance, P. von Aderkas and P.J. Charest. 1994. Transient chimeric gene expression in pollen of five conifer species following microparticle bombardment. Can. J. For. Res. 24: 2417-2423.

Ho, R.H. 1991. A guide to pollen- and seed-cone morphology of black spruce, white spruce, jack pine and eastern white pine for controlled pollination. Ontario Ministry of Natural Resources, Ontario Forest Research Institute. 31 pp.

Ho, R.H., and O. Hak. 1994. Optimum timing of gibberellin $A_{4/7}$ sprays to promote cone production in jack pine seedlings. New For. 8: 61-69.

Hodson, A.C., D.W. French, R.A. Jensen and R.J. Bartelt. 1982. The susceptibility of jack pine from Lake States seed sources to insects and diseases. USDA Forest Service North Central Forest Experiment Station Research Paper NC-225. 12 pp.

Hodson, A.C., D.W. French and R.A. Jensen. 1986. Role of insects and diseases in a jack pine provenance study. Great Lakes Entomol. 19: 239-247.

Hofgaard, A., J. Tardif and Y. Bergeron. 1999. Dendroclimatic response of *Picea mariana* and *Pinus banksiana* along a latitudinal gradient in the eastern Canadian boreal forest. Can. J. For. Res. 29: 1333–1346.

Hosie, R.C. 1979. Native trees of Canada, 8th ed. Fitzhenry & Whiteside Ltd., Don Mills, Ontario. 380 pp.

Houle, G., and L. Filion. 1993. Interannual variations in the seed production of *Pinus banksiana* at the limit of the species distribution in northern Quebec, Canada. Am. J. Bot. 80: 1242-1250.

Howse, G.M. 1984. Insect pests of jack pine: Biology, damage and control. Pp. 131-138 *in* Proceedings of Jack Pine Symposium. Canadian Forest Service, Great Lakes Forest Research Centre, COFJRC Symposium Proceedings O-P-12.

Hyun, J.O. 1979. Geographic variation of jack pine (*Pinus banksiana* Lamb.). Pp. 107-116 *in* Proceedings of the 13th Lake States Forest Tree Improvement Conference, August 17-18, 1977, University of Minnesota, St. Paul. USDA Forest Service North Central Forest Experiment Station General Technical Report GTR-NC-50.

Jacobson Jr., G.L., T. Webb III and E.C. Grimm. 1987. Patterns and rates of vegetation change during the deglaciation of eastern North America. Pp. 277-288 *in* W.F. Ruddiman and J.E. Wright Jr. (eds.), North America and adjacent oceans during the last deglaciation. Geographical Society of America, Boulder, Colorado, The Geology of North America K-3.

Jeffers, R.M. 1971. Research at the Institute of Forest Genetics. USDA Forest Service North Central Forest Experiment Station Research Paper NC-67. 31 pp.

Jeffers, R.M. 1972. Cone characteristics and seed yield in jack pine. Pp. 35-43 *in* Proceedings of the 19th Northeastern Forest Tree Improvement Conference, Maine.

Jeffers, R.M. 1978. Seed source-related eastern pineshoot borer incidence in jack pine. Silvae Genet. 27: 211-214.

Jeffers, R.M., and R.A. Jensen. 1980. Twenty-year results of the Lake States jack pine seed source study. USDA Forest Service North Central Forest Experiment Station Research Paper NC-181. 20 pp.

Jerome, C.A., and B.A. Ford. 2002. Comparative population structure and genetic diversity of *Arceuthobium americanum* (Viscaceae) and its *Pinus* host species: Insight into host–parasite evolution in parasitic angiosperms. Mol. Ecol. 11: 407-420.

Johnson, L.C., and W.B. Critchfield. 1978. The production of functional pollen and ovules by pine seedlings less than 1 year old. For. Sci. 24: 467-468.

Joyce, D., and P. Nitschke. 1993. An advanced generation breeding strategy for jack pine in Ontario. Pp. 13-24 *in* Y.S. Park and G.W. Adams (eds.), Breeding strategies of important tree species in Canada: Workshop proceedings. Natural Resources Canada, Canadian Forest Service, Maritimes Region Information Report M-X-186E/F. 54 pp.

Kelsall, J.P., E.S. Telfer and T.D. Wright. 1977. The effects of fire on the ecology of the boreal forest, with particular reference to the Canadian North: A review and selected bibliography. Canadian Wildlife Service Occasional Paper No. 32. 58 pp.

Keng, H., and E.L. Little Jr. 1961. Needle characteristics of hybrid pines. Silvae Genet. 10: 131-146.

Kenkel, N.C., M.L. Hendrie and I.E. Bella. 1997. A long-term study of *Pinus banksiana* population dynamics. J. Veg. Sci. 8: 241-254.

Kennedy, R.W. 1971. Influence of provenance and test location on wood formation in *Pinus banksiana* Lamb. seedlings. Silvae Genet. 20: 60-63.

Khasa, P.D., B. Hambling, G. Kernaghan, M. Fung and E. Ngimbi. 2002. Genetic variability in salt tolerance of selected boreal woody seedlings. For. Ecol. Manag. 165: 257-269.

King, J.P. 1966. Ten-year height growth variation in Lake States jack pine. Pp. 84-88 *in* Proceedings of the 2nd Genetics Workshop of the Society of American Foresters and the 7th Lake States Forest Tree Improvement Conference. USDA Forest Service North Central Forest Experiment Station Research Paper NC-6.

King, J.P. 1968. Seed source variation in tracheid length and specific gravity of five-year-old jack pine seedlings. Pp. 5-9 *in* Proceedings of the 8th Lake States Forest Tree Improvement Conference, 1967. USDA Forest Service North Central Forest Experiment Station Research Paper NC-23.

King, J.P. 1971. Pest susceptibility variation in Lake States jack pine seed sources. USDA Forest Service North Central Forest Experiment Station Research Paper NC-53. 12 pp.

King, J.P., and H. Nienstaedt. 1965. Variation in needlecast susceptibility among 29 jack pine seed sources. Silvae Genet. 14: 194-198.

Klein, J.I. 1982. Genetically improved jack pine for Manitoba and Saskatchewan. Environment Canada, Canadian Forestry Service, Northern Forest Research Centre, Forest Management Note No. 18. 4 pp.

Klein, J.I. 1989a. Jack pine family selection in Saskatchewan based on 10-year measurement. Northern Forest Research Centre, Forest Management Note No. 46. 7 pp.

Klein, J.I. 1989b. Multiple-trait combined selection in jack pine family-test plantations using best linear prediction. Silvae Genet. 44: 362-375.

Klein, J.I. 1990. Survival and growth of jack pine provenances in Manitoba. Forestry Canada, Northwest Region, Forest Management Note No. 50. 6 pp.

Klein, J.I. 1995. Effects of incomplete block design and stand structure on heritability of growth in a jack pine family test in Manitoba. Can. J. For. Res. 19: 1044-1050.

Klein, J.I. 1998. A plan for advanced-generation breeding of jack pine. For. Genet. 5: 73-83.

Klein, J.I., C.G. Davidson and R.D. Browne. 1995. Asexual propagation for jack pine genetic improvement (abstract). Page 83 in J. Lavereaus (ed.), Proceedings of the 25th Meeting of the Canadian Tree Improvement Association, Part 2, 28 August to 1 September 1995, Victoria, B.C. Natural Resources Canada.

Knowles, P. 1985. Comparison of isozyme variation among natural stands and plantations: Jack pine and black spruce. Can. J. For. Res. 15: 902-908.

Kormuťák, A., R. Matúsová, A. Szmidt and D. Lindgren. 1993. Karyological, anatomical and restriction fragment length polymorphism characteristics of the interspecific hybrid *Pinus banksiana* × *Pinus contorta*. Biológia (Bratislava) 48: 95-100.

Kral, R. 1993. *Pinus* Linnaeus. Pp. 373-398 in Flora of North America Editorial Committee (ed.), Flora of North America North of Mexico, Vol. 2: Pteridophytes and Gymnosperms. Oxford University Press, New York.

Lavigne, M.B. 1996. Comparing stem respiration and growth of jack pine provenances from northern and southern locations. Tree Physiol. 16: 847-852.

Law, K.N., and J.L. Valade. 1994. Status of the utilization of jack pine (*Pinus banksiana*) in the pulp and paper industry. Can. J. For. Res. 14: 2078-2084.

Little Jr., E.L. 1971. Atlas of United States trees, Vol. 1, Conifers and important hardwoods. USDA Miscellaneous Publication No. 1146. 9 pp. + 200 maps.

Little Jr., E.L., and W.B. Critchfield. 1969. Subdivisions of the genus *Pinus* (pines). USDA Forest Service Miscellaneous Publication No. 1144. 51 pp.

Little, M.T. 1984. Status report on the management of jack pine in the Province of Saskatchewan. Pp. 20-28 *in* Proceedings of Jack Pine Symposium. Canadian Forest Service, Great Lakes Forest Research Centre, COFJRC Symposium Proceedings O-P-12.

Logan, K.T. 1971. Monthly variations in photosynthetic rate of jack pine provenances in relation to their height. Can. J. For. Res. 1: 256-261.

Lotan, J.E. 1967. Early performance of *Pinus contorta* × *banksiana* hybrids. USDA Forest Service Intermountain Forest and Range Experiment Station Research Note INT-57. Ogden, Utah. 8 pp.

Magnussen, S. 1990. Alternative analyses of parent-offspring correlations for stem straightness in jack pine. Silvae Genet. 39: 237-243.

Magnussen, S., and C.T. Keith. 1990. Genetic improvement of volume and wood properties of jack pine: Selection strategies. For. Chron. 66: 281-286.

Magnussen, S., and C.W. Yeatman. 1979. Height growth components in inter- and intra-provenance jack pine families. Can. J. For. Res. 9: 962-972.

Magnussen, S., and C.W. Yeatman. 1987. Early testing of jack pine. I. Phenotypic response to spacing. Can. J. For. Res. 17: 453-459.

Magnussen, S., and C.W. Yeatman. 1988a. Height growth and survival of within- and between-provenance crosses in jack pine. Can. J. For. Res. 18: 1145-1151.

Magnussen, S., and C.W. Yeatman. 1988b. Provenance hybrids in jack pine: 15-year results in eastern Canada. Silvae Genet. 37: 206-218.

Magnussen, S., and C.W. Yeatman. 1990. Predictions of genetic gain from various selection methods in open pollinated *Pinus banksiana* progeny trials. Silvae Genet. 39: 140-153.

Maley, M.L., and W.H. Parker. 1993. Phenotypic variation in cone and needle characters of *Pinus banksiana* (jack pine) in northwestern Ontario. Can. J. Bot. 71: 43-51.

Marles, R.J., C. Clavelle, L. Monteleone, N. Tays and D. Burns. 2000. Aboriginal plant use in Canada's northwest boreal forest. Natural Resources Canada, Canadian Forest Service / University of British Columbia Press, Vancouver, B.C. 368 pp.

Matyas, C., and C.W. Yeatman. 1992. Effect of geographical transfer on growth and survival of jack pine (*Pinus banksiana* Lamb.) populations. Silvae Genet. 41: 370-376.

McAfee, B.J., E.E. White, L.E. Pelcher and M.S. Lapp. 1993. Root induction in pine (*Pinus*) and larch (*Larix*) spp. using *Agrobacterium rhizogenes*. Plant Cell, Tissue Organ Cult. 34: 53-62.

McGauley, B.H., and H.L. Gross. 1984. Jack pine diseases in Ontario. Pp. 131-138 *in* Proceedings of Jack Pine Symposium. Canadian Forest Service, Great Lakes Forest Research Centre, COFJRC Symposium Proceedings O-P-12.

McLeod, T.K., and G.M. MacDonald. 1997. Postglacial range expansion and population growth of *Picea mariana*, *Picea glauca* and *Pinus banksiana* in the western interior of Canada. J. Biogeogr. 24: 865-881.

Mergen, F., and J. Worrall. 1965. Effect of environment and seed source on mineral content of jack pine seedlings. For. Sci. 11: 393-400.

Mergen, F., J. Worrall and G.M. Furnival. 1967. Genotype–environment interactions in 50 sources of jack pine seedlings. Pp. 459-466 *in* IUFRO Congress, Munich, Papers III Section 22-AG22/24.

Miksche, J.P. 1968. Quantitative study of intraspecific variation of DNA per cell in *Picea glauca* and *Pinus banksiana*. Can. J. Genet. Cytol. 10: 590-600.

Mirov, N.T. 1956. Composition of turpentine of lodgepole × jack pine hybrids. Can. J. Bot. 34: 443-457.

Mirov, N.T., and J. Hasbrouck. 1976. The story of pines. Indiana University Press, Bloomington, Indiana. 148 pp.

Misenti, T.L., and D.H. DeHayes. 1988. Genetic diversity of marginal vs. central populations of pitch pine and jack pine. Pp. 63-72 *in* M.E. Demerritt (ed.), Proceedings of the 31st Northeastern Tree Improvement Conference and the 6th North Central Tree Improvement Conference, Pennsylvania State University.

Morgenstern, E.K., and A.H. Teich. 1969. Phenotypic stability of height growth of jack pine provenances. Can. J. Genet. Cytol. 11: 110-117.

Morris, D.M., W.H. Parker and R. Seabrook. 1992. Some considerations when selecting young jack pine families using growth and form traits. Can. J. For. Res. 22: 429-435.

Moss, E.H. 1949. Natural pine hybrids in Alberta. Can. J. Res. Sect. C Bot. Sci. 27: 218-229.

Nkongolo, K.K., and W.S. Gratton. 2001. Genetic variation in *Pinus banksiana* from Sudbury (Ontario, Canada) region. Silvae Genet. 50: 122-127.

Nozzolillo, C., P. Isabelle, Ø.M. Anderson and M. Abou-Zaid. 2002. Anthocyanins of jack pine (*Pinus banksiana*) seedlings. Can. J. Bot. 80: 796-801.

Okwuagwu, C.O., and R.P. Guries. 1980. Estimates of general and specific combining ability and heritability for juvenile wood specific gravity and tracheid length in jack pine. Pp. 128-137 *in* Proceedings of the 27th Northeastern Forest Tree Improvement Conference, Burlington, Vermont.

Owens, J.N., and M.D. Blake. 1985. Forest tree seed production. Canadian Forestry (Forest???) Service, Petawawa National Forest Institute Information Report P1-X-S, Chalk River, Ontario. 161 pp.

Park, Y.S., J.M. Bonga and I. MacEacheron. 2000. Somatic embryogenesis of conifers, stability of embryogenic clones, and clonal forestry. Pp. 40 *in* Proceedings of the 27th Meeting of the Canadian Tree Improvement Association, Sault Ste. Marie, Ontario, Part 1.

Park, Y.S., J.D. Simpson, D.P. Fowler and E.K. Morgenstern. 1989. A selection index with desired gains to rogue jack pine seedling seed orchards. Forestry Canada, Maritimes Region Information Report M-X-176. 18 pp.

Parker, W.H., and A. van Niejenhuis. 1996. Seed zone delineation for jack pine in the former Northwest Region of Ontario using short-term testing and Geographic Information Systems. Northern Ontario Development Agreement / Northern Forestry Program, NODA/NFP Technical Report TR-35. 34 pp.

Pellerin, S., and C. Lavoie. 2003. Recent expansion of jack pine in peatlands of southeastern Québec: A paleoecological study. Écoscience 10: 247-257.

Pelletier, G., and S. Laliberté. 2000. Effect of embryo orientation on the developmental sequence of adventitious organogenesis in jack pine (*Pinus banksiana* Lamb.). Can. J. Bot. 78: 1348-1360.

Perry, D.J., and J. Bousquet. 1998. Sequence-tagged-site (STS) markers of arbitrary genes: The utility of black spruce-derived STS primers in other conifers. Theor. Appl. Genet. 97: 735-743.

Peterson, R. 1980. The pine tree book. Brandywine Press, Inc., New York. 138 pp. + glossary.

Potapova, S.A. 1988. The propagation of introduced species of *Pinus* by winter cuttings. (Summary). Byulleten' Glavnogo Botanicheskogo Sada 148: 35-37.

Price, R.A., A. Liston and S.H. Strauss. 1998. Phylogeny and systematics of *Pinus*. Pp. 49-68 in Richardson, D.M. (ed.), Ecology and biogeography of *Pinus*. Cambridge University Press, New York.

Rake, A.V., J.P. Miksche, R.B. Hall and K.M. Hansen. 1980. DNA reassociation kinetics of four conifers. Can. J. Genet. Cytol. 22: 69-79.

Rauf, A., R.A. Cecich and D.M. Benjamin. 1984. Conelet abortion in jack pine caused by *Platylygus luridus* (Hemiptera: Miridae). Can. Entomol. 116: 1213-1218.

Rauf, A., D.M. Benjamin and R.A. Cecich. 1985. Insects affecting seed production of jack pine, and life tables of conelet and cone mortality in Wisconsin. For. Sci. 31: 271-281.

Rehfeldt, G.E., and J.E. Lotan. 1970. *Pinus contorta* × *banksiana* hybrids tested in northern Rocky Mountains. USDA Forest Service Intermountain Forest and Range Experiment Station Research Note INT-126. Ogden, Utah. 7 pp.

Richardson, D.M. (ed.). 1998. Ecology and biogeography of *Pinus*. Cambridge Univers. Press, New York. 527 pp.

Riemenschneider, D.E. 1985. Water stress promotes early flowering in jack pine. USDA Forest Service North Central Forest Experiment Station Research Note NC-331. 3 pp.

Riemenschneider, D.E. 1988. Heritability, age-age correlations, and inferences regarding juvenile selection in jack pine. For. Sci. 34: 1076-1082.

Righter, F.I. 1939. Early flower production among the pines. J. For. 37: 935-938.

Roe, E.I. 1963. Seed stored in cones of some jack pine stands, northern Minnesota. USDA Forest Service Lake States Forest Experiment Station, St. Paul, Minnesota; Research Paper LS-1. 14 pp.

Ross, H.A., and J.L. Hawkins. 1986. Genetic variation among local populations of jack pine (*Pinus banksiana*). Can. J. Genet. Cytol. 28: 453-458.

Roussy, A.M., and P.G. Kevan. 2000. How accessible are receptive megastrobili to pollen? The example of jack pine (*Pinus banksiana*). Am. J. Bot. 87: 215-220.

Rudolf, P.O. 1958. Silvical characteristics of jack pine. USDA Forest Service Lake States Forest Experiment Station, Station Paper No. 61.

Rudolf, P.O. 1963. Botanical and commercial range of jack pine in the Lake States. USDA Forest Service Lake States Forest Experiment Station Research Note LS-15.

Rudolf, P.O. 1965. Jack pine (*Pinus banksiana* Lamb.). *In* Fowells, H.A. (compiler) Silvics of forest trees of the United States. pp. 338-354. USDA Forest Service Agriculture Handbook No. 271. 762 pp.

Rudolph, T.D. 1964. Lammas growth and prolepsis in jack pine in the Lake States. Society of American Foresters, Washington, D.C., Forest Science Monograph 6, 70 pp.

Rudolph, T.D. 1966a. Segregation for chlorophyll deficiencies and other phenodeviants in the X1 and X2 generations of irradiated jack pine. Pp. 18-23 *in* Proceedings of the 2nd Genetics Workshop of the Society of American Foresters and the 7th Lake States Forest Tree Improvement Conference, USDA Forest Service North Central Forest Experiment Station Research Paper NC-6.

Rudolph, T.D. 1966b. Stimulation of earlier flowering and seed production in jack pine seedlings through greenhouse and nursery culture. Pp. 80-83 *in* Proceedings of the 2nd Genetics Workshop of the Society of American Foresters and the 7th Lake States Forest Tree Improvement Conference, USDA Forest Service North Central Forest Experiment Station Research Paper NC-6.

Rudolph, T.D. 1979a. Female strobili on 12-month-old jack pine. Tree Planters' Notes 30: 24-26.

Rudolph, T.D. 1979b. Seed production in the first eight years and frequency of natural selfing in a simulated jack pine seedling seed orchard. Pp. 33-47 *in* Proceedings of the 13th Lake States Forest Tree Improvement Conference, August 17-18, 1977, University of Minnesota, St. Paul. USDA Forest Service North Central Forest Experiment Station General Technical Report GTR-NC-50.

Rudolph, T.D. 1981a. Four-year height growth variation among and within S0, S1 × S1, S1 open-pollinated, and S2 inbred jack pine families. Can. J. For. Res. 11: 654-661.

Rudolph, T.D. 1981b. Variation in early flowering of inbred jack pine families. Pp. 119-129 *in* W.T. Bagley and D.F. Van Haverbeke (co-chairs), Proceedings of the 2nd North Central Tree Improvement Conference, Nebraska.

Rudolph, T.D. 1984. Status report on the management of jack pine in the Lake States. Pp. 38-46 *in* Proceedings of Jack Pine Symposium. Canadian Forest Service, Great Lakes Forest Research Centre, COJFRC Proceedings O-P-12.

Rudolph, T.D., and P.R. Laidly. 1990. *Pinus banksiana* Lamb. – jack pine. Pp. 280-293 *in* R.M. Burns and B.H. Honkala (eds.), Silvics of North America, Vol. 1, Conifers. USDA Forest Service Agriculture Handbook No. 654.

Rudolph, T.D., and H. Nienstaedt. 1962. Polygenic inheritance of resistance to winter injury in jack pine – lodgepole pine hybrids. J. For. 60: 138-139.

Rudolph, T.D., and H. Nienstaedt. 1964. Rooting, shoot development, and flowering of jack pine needle fascicles. Silvae Genet. 13: 118-123.

Rudolph, T.D., and C.W. Yeatman. 1982. Genetics of jack pine. USDA Forest Service Research Paper WO-38. 60 pp.

Rudolph, T.D., H.C. Kang and R.P. Guries. 1989. Realised genetic gain for 2nd-year height in jack pine. Can. J. For. Res. 19: 707-714.

Saenz-Romero, C., and R.P. Guries. 2002. Landscape genetic structure of *Pinus banksiana*: Seedling traits. Silvae Genet. 51: 26-35.

Saenz-Romero, C., R.P. Guries and A. Monk. 2001. Landscape genetic architecture of *Pinus banksiana*: Allozyme variation. Can. J. Bot. 79: 871-878.

Sande, D., and L. Corriveau. 2000. Weyerhaeuser – Tree improvement in Saskatchewan. Page 87 *in* Proceedings of the 27th Meeting of the Canadian Tree Improvement Assocociation, Sault Ste. Marie, Ontario, Part 1.

Saylor, L.C. 1972. Karyotype analysis of the genus *Pinus* – subgenus *Pinus*. Silvae Genet. 19: 155–163.

Schantz-Hansen, T., and R.A. Jensen. 1952. The effect of source of seed on growth of jack pine. J. For. 50: 539-544.

Schnare, P.D. 1969. A provenance study of jack pine in central Missouri. M.S. thesis, University of Missouri, School of Forestry. 99 pp.

Schoenike, R.E. 1976. Geographical variation in jack pine (*Pinus banksiana*). University of Minnesota Agricultural Experiment Station Technical Bulletin 304, Forestry Series 21. 47 pp.

Simpson, J.D. 1997. The New Brunswick tree improvement program. J. For. 95: 12-14.

Simpson, J.D., and B. Daigle. 1998. National Forest Genetic Resources Centre. Pp. 47-48 *in* J.D. Simpson (ed.), Proceedings of the 26th Meeting of the Canadian Tree Improvement Association, Ste. Foy, Québec, Part 1.

Simpson, J.D., and V.C. Steel. 1995. New Brunswick Tree Improvement Council update. Pp. 151-153 *in* J. Lavereaus (ed.), Proceedings of the 25th Meeting of the Canadian Tree Improvement Association, Victoria, B.C., Part 1.

Simpson, J.D., and K. Tosh. 1997. The New Brunswick Tree Improvement Council is 20 years old. For. Chron. 73: 572-577.

Sittmann, K., and H. Tyson. 1971. Estimates of inbreeding in *Pinus banksiana*. Can. J. Bot. 49: 1241-1245.

Skeates, D.A. 1976. Discontinuity in growth potential of jack pine in Ontario: A 20-year provenance assessment. For. Chron. 55: 137-141.

Snyder, T.P., D.A. Stewart and A.F. Strickler. 1985. Temporal analysis of breeding structure in jack pine (*Pinus banksiana* Lamb.). Can. J. For. Res. 15: 1159-1166.

Sprackling, J.A., and R.A. Read. 1975. Jack pine provenance study in eastern Nebraska. USDA Forest Service Research Paper RM-143. 8 pp.

Steiner, K.C. 1979. Patterns of variation in bud-burst timing among populations in several *Pinus* species. Silvae Genet. 28: 185-194.

Stine, R.A., L.K. Miller, G. Wyckoff, B. Li and B.L. Li. 1995. A comprehensive tree improvement plan for Minnesota. University of Minnesota, Department of Forest Resources Staff Paper Series No. 102. 24 pp.

St-Pierre, H., R. Gagnon and P. Bellefleur. 1992. Régénération après feu de l'épinette noire (*Picea mariana*) et du pin gris (*Pinus banksiana*) dans la forêt boréale. Can. J. For. Res. 22: 474–481.

Strong, T.F., and D.F. Grigal. 1987. Site and seed source influence jack pine yields in the Lake States. Can. J. For. Res. 17: 705-711.

Sweet, G.B., and I.J. Thulin. 1963. The performance of six provenances of *Pinus banksiana* in a 7-year-old trial in New Zealand. Forest Research Institute, Rotorua, New Zealand; Research Leaflet No. 2. 3 pp.

Tautorus, T.E., F. Bekkaoui, M. Pilon, R.S.S. Datla, W.L. Crosby, L.C. Fowke and D.I. Dunstan. 1989. Factors affecting transient gene expression in electroporated black spruce (*Picea mariana*) and jack pine (*Pinus banksiana*) protoplasts. Theor. Appl. Genet. 78: 531-536.

Teich, A.H. 1970. Cone serotiny and inbreeding in natural populations of *Pinus banksiana* and *Pinus contorta*. Can. J. Bot. 48: 1805-1809.

Thomas, P.A., and R.W. Wein,. 1985. Delayed emergence of four conifer species on postfire seedbeds in eastern Canada. Can. J. For. Res. 15: 727-729.

Todhunter, M.N., and R.B. Polk. 1981. Seed and cone production in a clonal orchard of jack pine (*Pinus banksiana*). Can. J. For. Res. 11: 512-516.

Tosh, K.J., and B.G. McInnis. 2000. New Brunswick Tree Improvement Council update. Pp. 28-30 *in* Proceedings of the 27th Meeting of the Canadian Tree Improvement Association, Sault Ste. Marie, Ontario, Part 1.

U.S. Geological Survey. 2002. Digital representations of tree species range maps. U.S. Department of the Interior, Geological Survey, Earth Surface Processes. http://climchange.cr.usgs.gov/data/atlas/little/

van Niejenhuis, A., and W.H. Parker. 1996. Adaptive variation in jack pine from north central Ontario determined by short-term common garden tests. Can. J. For. Res. 26: 2006-2014.

Villeneuve, M., E.K. Morgenstern and L.P. Sebastian. 1987a. Estimation of wood density in family tests of jack pine and black spruce using the Pilodyn tester. Can. J. For. Res. 17: 1147-1149.

Villeneuve, M., E.K. Morgenstern and L.P. Sebastian. 1987b. Variation patterns and age relationships of wood density in families of jack pine and black spruce. Can. J. For. Res. 17: 1219-1222.

Wagner, D.B., D.R. Govindaraju and B.P. Dancik. 1988. Chloroplast DNA polymorphism in a sympatric region. Pp. 75-78 *in* W.M. Cheliak and A.C. Yapa (eds.), Molecular genetics of forest trees. Proceedings 2nd IUFRO Working Party on Molecular Genetics, Petawawa National Forest Institute, Chalk River, Ontario.

Wagner, D.B., Z.-X. Sun, D.R. Govindaraju and B.P. Dancik. 1991. Spatial patterns of chloroplast DNA and cone morphology variation within populations of a *Pinus banksiana – Pinus contorta* sympatric region. Am. Nat. 138: 156-170.

Wagner, D.B., G.R. Furnier, M.A. Saghai-Maroof, S.M. Williams, B.P. Dancik and R.W. Allard. 1987. Chloroplast DNA polymorphisms in lodgepole and jack pines and their hybrids. Proc. Natl. Acad. Sci. U.S. Am. 84: 2097-2100

Wheeler, N.C., and R.P. Guries. 1987. A quantitative measure of introgression between lodgepole and jack pines. Can. J. Bot. 65: 1876-1885.

Wu, H.X., and C.C. Ying. 1998. Stability of resistance to western gall rust and needle cast in lodgepole pine provenances. Can. J. For. Res. 28: 439-449.

Wu, H.X., C.C. Ying and J.A. Muir. 1996. Effect of geographic variation and jack pine introgression on disease and insect resistance in lodgepole pine. Can. J. For. Res. 26: 711-726.

Wyman, J., S. Laliberte and M.-F. Tremblay. 1997. Nuclear DNA content variation in seeds from 22 half-sib families of jack pine (*Pinus banksiana*, Pinaceae). Am. J. Bot. 84: 1351-1361.

Xie, C.Y., and P. Knowles. 1991. Spatial genetic substructure within natural populations of jack pine (*Pinus banksiana*). Can. J. Bot. 69: 547-551.

Yang, R.-C., N.K. Dhir and L.K. Barnhardt. 1998. Comparative assessment of genetic variation of young high-elevation lodgepole pine for height and western gall rust resistance across two sites in Alberta. Can. J. For. Res. 28: 478-484.

Yang, R.-C., Z. Ye, and Y. Hiratsuka. 1999. Susceptibility of *Pinus contorta – Pinus banksiana* complex to *Endocronartium harknessii*: Host-pathogen interactions. Can. J. Bot. 77: 1035-1043.

Yang, R.-C., N.K. Dhir, F.C. Yeh and Y. Hiratsuka. 1997. Geographic variation in susceptibility of Alberta lodgepole pine to western gall rust. Can. J. For. Res. 27: 1398-1405.

Ye, T.Z., R.-C. Yang and F.C. Yeh. 2002. Population structure of a lodgepole pine (*Pinus contorta*) and jack pine (*Pinus banksiana*) complex as revealed by random amplified polymorphic DNA. Genome 45: 530-540.

Yeatman, C.W. 1966. Germinant size of jack pine in relation to seed size and geographic origin. Pp 28-36 *in* Proceedings of the 2nd Genetics Workshop of the Society of American Foresters and the 7th Lake States Forest Tree Improvement Conference. USDA Forest Service North Central Forest Experiment Station Research Paper NC-6.

Yeatman, C.W. 1967. Biogeography of jack pine. Can. J. Bot. 45: 2201-2211.

Yeatman, C.W. 1974. The jack pine genetics program at Petawawa Forest Experiment Station 1950-1970. Canadian Forest Service Publication 1331. 30 pp.

Yeatman, C.W. 1984. The genetic basis of jack pine management. Pp. 9-19 *in* Proceedings of Jack Pine Symposium. Canadian Forest Service, Great Lakes Forest Research Centre, COFJRC Symposium Proceedings O-P-12.

Yeatman, C.W., and M.J. Holst. 1967. Correlations between controlled-environment and nursery-grown seedlings of jack pine provenances. Pp. 24-30 *in* Proceedings of the 14th Northeastern Forest Tree Improvement Conference, Toronto, Ontario.

Yeatman, C.W., and M.J. Holst. 1972. Hardiness of jack pine hybrids and provenances in the boreal forest. For. Chron. 48: 30-31.

Yeatman, C.W., and E.K. Morgenstern. 1979. Seed source variation. Pp. 48-61 *in* J.B. Scarratt (coord.), Tree Improvement Symposium. Canadian Forest Service, Sault Ste. Marie, Ontario, COJFRC Symposium Proceedings O-P-7.

Yeatman, C.W., and A.H. Teich. 1969. Genetics and breeding of jack and lodgepole pines in Canada. For. Chron. 45: 428-433.

Zavarin, E., W.B. Critchfield and K. Snajberk. 1969. Turpentine composition of *Pinus contorta* × *Pinus banksiana* hybrids and hybrid derivatives. Can. J. Bot. 47: 1443-1453.

Zhang, J.W., and B.M. Cregg. 1996. Variation in stable carbon isotope discrimination among and within exotic conifer species grown in eastern Nebraska, USA. For. Ecol. Manag. 83: 181-187.

Zhang, S.Y. 1995. Forintek's wood quality and end-use studies related to tree improvement programs. Pp. 129-132 *in* J. Lavereaus (ed.), Proceedings of the 25th Meeting of the Canadian Tree Improvement Association, Victoria, B.C., Part 1.

Zhang, S.Y., and Y.H. Chui. 1996. Selecting dry fiber weight for higher and better quality jack pine fiber production. Wood Fiber Sci. 28: 146-152.

Zsuffa, L. 1974. Rooting of jack pine (*Pinus banksiana* Lamb.) cuttings. Can. J. For. Res. 4: 557-561.

Section 3.
Native north american larches: subalpine larch (*Larix lyallii*), western larch (*L. occidentalis*), and tamarack (*L. laricina*)

Preamble:

Each of the three North American larch species is usually discussed separately in each section and subsection of this Consensus Document in the following order: subalpine larch (*Larix lyallii*), western larch (*Larix occidentalis*), and tamarack (*Larix laricina*).

1. Taxonomy

Larch forests essentially encircle the colder temperate Northern Hemisphere. Within this area, the larch genus (*Larix*) is represented by 10-15 species and some subspecies or varieties as well as natural hybrids (Schmidt, 1995; Semerikov and Lascoux 2003; Semerikov *et al*, 2003). Ten species usually recognised are the North Eurasian *Larix decidua*, *L. sibirica* (synonym *L. russica*), *L. gmelinii* (including *L. cajanderi*, *L. dahurica*, and possibly *L. olgensis*), and *L. kaempferi* (synonym *L. leptolepis*); the South Asian *L. griffithiana*, *L. mastersiana*, and *L. potaninii*; and the North American *L. laricina*, *L. lyallii*, and *L. occidentalis*. All true larches are in the genus *Larix* Mill., a deciduous needle-leaf lineage in the gymnosperm family Pinaceae. Larch taxonomy has had limited overall attention. This is reflected in a lack of consensus about what constitutes a larch species or subspecies (or botanical variety), and about the phylogenetic relationships among species (Semerikov *et al*, 2003). The proposed division of *Larix* into two sections based largely on cone morphology (Vidakovic, 1991) is not supported by studies of chloroplast DNA variation (Qian *et al*, 1995), nuclear internal transcribed spacer (ITS) region (Gernandt and Liston, 1999), amplified fragment length polymorphisms (AFLPs) (Semerikov *et al*, 2003), or allozyme variation (Semerikov and Lascoux, 1999; Semerikov *et al*, 1999). An early phylogenetic separation (perhaps in the late Tertiary) occurred between the North American and the Eurasian species (Gernandt and Liston, 1999; Semerikov and Lascoux, 1999; Semerikov *et al*, 2003). The native range of each of the three North American larches – *Larix lyallii* Parl. (subalpine larch), *L occidentalis* Nutt. (western larch), and *L. laricina* (Du Roi) K. Koch (tamarack or North American larch) – is well defined with nearly no overlap between them. The disjunct western portion of the range of *L. laricina* (Figure 3) was recognised as the separate species *L. alaskensis* W. Wight (*e.g.* Hosie, 1979), or as the variety *L. laricina* var. *alaskensis* (W. Wight) Raup (Stipanicic, 1975) based on cone and needle morphology. This distinction was not recognised by Viereck and Little (1972), and species or varietal status is not supported by the degree of morphological and anatomical differentiation of the Alaskan tamarack populations (Parker and Dickinson, 1990; Parker, 1993).

The three North American species of *Larix* can be morphologically differentiated as follows: subalpine larch has 4-angled rather than somewhat 3-angled needles in cross section, and its new-growth twigs are densely covered with white cottony hairs. Compared to the other two larches, tamarack has smaller seed cones (1-2 cm long) with fewer (10-30) scales and bracts shorter than the scales; western larch (which has reddish-brown bark) has cones about 2.5-4.5 cm long and the bracts are extended, whereas subalpine larch (which has grayish bark) has cones often still larger (about 4-5 cm long) that appear bristly because the bracts extend farther beyond the scales.

2. Natural distribution

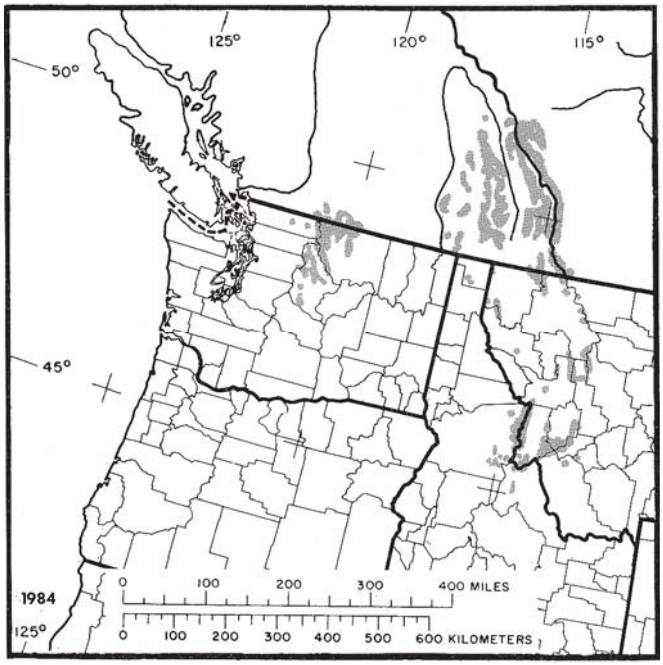
For about 200 years, attempts have been made to identify superior non-native species of larch for reforestation. As a result, plantation of exotic larches can be observed at many locations in the world, particularly in Europe and eastern North America (Krüssmann, 1985). The Japanese larch (*L. kaempferi*) is cultivated in many European plantations because of its more rapid growth than the native larches. Although introduced to Europe and Asia for testing, western larch has not been grown as a timber crop species outside its native range; it is grown ornamentally in arboreta and parks. Sometimes *L. decidua* (European larch) spreads locally from plantings in northeastern North America.

2.1 Subalpine larch

Subalpine larch is a continental, subalpine to boreal, western North American (prevailingly Cordilleran) species (Klinka *et al*, 2000) (Figure 1). It occupies a remote and rigorous environment, growing in and near the treeline. Although occurring in both the Rocky Mountains and the Cascade Range farther to the west (Little, 1979), the two distributions are separated by 200 km at their closest points, in Canada in southern British Columbia (Arno, 1990).

In British Columbia and Alberta, subalpine larch is common along the Continental Divide and adjacent ranges, and in the Purcell Range and southern Selkirk Range (Klinka *et al*, 2000). In the Rocky Mountains, subalpine larch extends from the Salmon River Mountains of central Idaho (latitude 45°30' N) northward to Lake Louise in Banff National Park, Alberta (latitude 51°30' N). Within this distribution, subalpine larch is common in the highest areas of the Bitterroot, Anaconda-Pintler, Whitefish, and Cabinet ranges of western Montana (Arno and Habeck, 1972). In the Cascades, subalpine larch is found principally east of the Cascade Divide and extends from the Wenatchee Mountains in central Washington northward to British Columbia. It spans an elevational range of 1520 to 3020 m (Arno, 1990).

Figure 1. The native range of subalpine larch

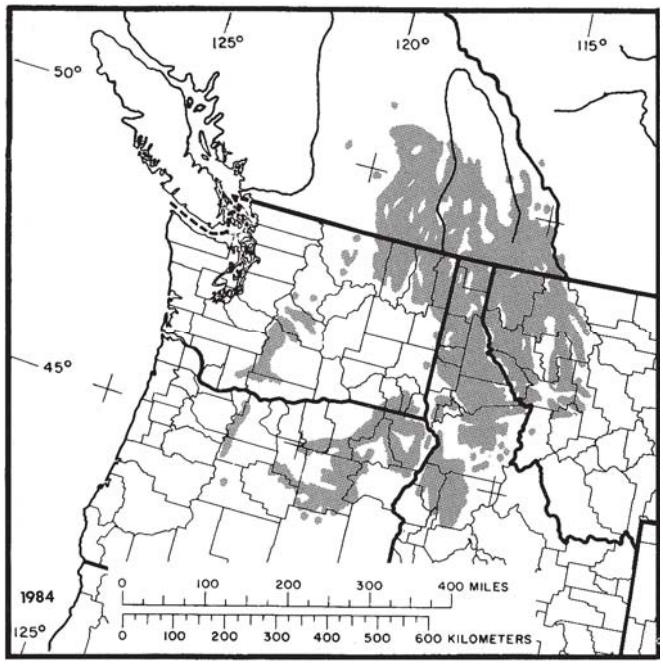


Source: Arno, 1990

2.2 Western larch

Western larch, a western North American (predominantly Cordilleran) species (Klinka *et al*, 2000), has a relatively moderate native range (Figure 2). It grows in southeastern British Columbia, northeastern Washington, the Upper Columbia River Basin of northwestern Montana and across northern and west-central Idaho; and farther westward in the Wallowa and Blue Mountains of southeastern Washington and northeastern Oregon, to the eastern slopes of the Cascade Mountains in north-central Oregon and central Washington (Schmidt and Shearer, 1990). Western larch has an elevational range of around 1500 m, from approximately 500 to 2000 m (Rehfeldt, 1995b).

Figure 2. The native range of western larch



Source: Schmidt and Schearer, 1990

2.3 Tamarack

Tamarack is a transcontinental North American species with one of the widest ranges of all North American conifers (Klinka *et al*, 2000) (Figure 3). Its northern range limit extends in Canada from Newfoundland and Labrador westward along the northern treeline and across the Continental Divide in northern Yukon Territory to the Mackenzie River drainage. The southern limit is from Maine through northern Connecticut, New Jersey, Pennsylvania, and Ohio, across the lake states, then from Manitoba through central Alberta to northern British Columbia (Johnston, 1990). Its farthest south populations occur locally in the mountains of northern West Virginia and adjacent western Maryland. A major disjunct portion of the western range of tamarack is found in the interior of Alaska, in the Yukon and Kuskokwim river basins between the Brooks Range and the Alaska Range to the south; and three small areas are near or on the Alaska-Yukon border (Viereck and Little, 1972). In the eastern portion of its range, it grows from sea level to 1220 m elevation, while in the western portion of the range it is found between 180 and 520 m (Johnston, 1990).

Figure 3. The native range of tamarack



Source: Johnston, 1990

3. Reproductive biology

3.1. Reproductive development

3.1.1. Subalpine larch

Like all members of the genus *Larix*, subalpine larch is monoecious, with male and female strobili borne separately on spur shoots scattered among leaf-bearing spur shoots. Buds containing male and female strobili begin to swell in late May. Pollen is released from the small, yellowish male strobili and wind dispersed in June, when there is often still snow on the ground (Arno, 1970; Richards, 1981). Female strobili mature by September into 4-5 cm purplish cones. Strobili can be damaged by frost, and this may be a cause of low seed production in most years. The reproductive cycle of subalpine larch has not been studied in detail, and factors limiting pollination, fertilisation, and seed development are not well understood (Schopmeyer, 1974).

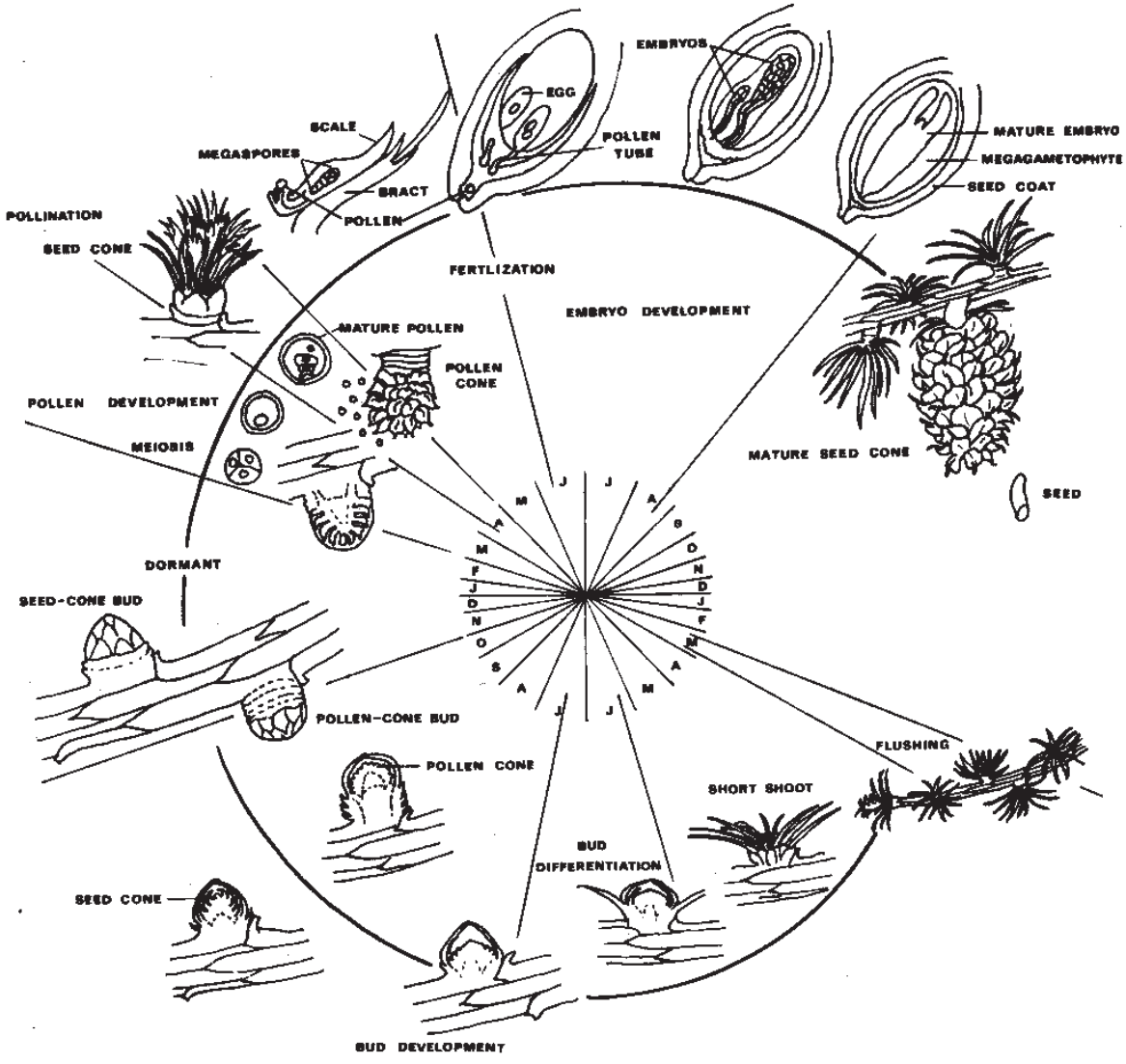
3.1.2. Western larch

Western larch is also monoecious; male and female strobili develop throughout the crown. Reproductive buds are found at the end of short spur shoots. Buds differentiate in June and July, and reproductive and vegetative buds can be distinguished early in the fall, about a year before subsequent cone crops mature (Schmidt and Shearer, 1990). Reproductive buds are larger than vegetative buds. Staminate buds are usually about one and one-half to two times longer than wide, whereas ovulate buds are globose (Figure 4). Buds and strobili can be sampled in fall to predict larch seed crops (Roe, 1966).

Pollen and seed strobili appear several days before vegetative buds open, which typically occurs between mid-April and mid-May (Schopmeyer, 1974; Owens and Molder, 1979b; Owens, 1995). Red or green female strobili are generally conspicuous. Pollination occurs in late May and early June. Following fertilisation, cones complete their development in that same season and mature by mid- to late-August of the same year, reaching 2.5 to 4.5 cm in length (Owens and Molder, 1979a, b; Schmidt and Lotan, 1980).

Stem injection of gibberellin $A_{4/7}$ in May or June increases both pollen and seed cone production (Ross, 1991; Eysteinsson and Greenwood, 1995; Shearer *et al*, 1999). Protocols for *in vitro* germination of western larch pollen have been developed (Dumont-BéBoux *et al*, 2000). The detailed documentation of the reproductive cycle of western larch by Owens and Molder (1979a, b) – illustrated in Figure 4 – is thought to hold for other species of *Larix* as well, albeit with some differences in timing (Owens, 1995).

Figure 4. The 2-year reproductive cycle of western larch



Source: Owens, 1995

3.1.3. Tamarack

Like other members of this genus, tamarack is monoecious with small, solitary male and female strobili interspersed with needles. Yellow male strobili are borne mainly on 1- or 2-year-old spur shoots. The reddish female strobili are borne most commonly on 2- to 4-year-old shoots. On open-grown trees, cones are borne on all parts of the crown. Ripe cones are brown, oblong to ovoid, and 1.3 to 1.9 cm long (Johnston, 1990). Reproductive buds on tamarack generally flush in Ontario and the Lake States from April to May, and in the interior of Alaska from mid- to late May. Seed cones generally ripen in Ontario and the Lake States in August and September (Schopmeyer, 1974).

3.2. Mating system and gene flow

3.2.1. Subalpine larch

Gene flow is likely less in this species than in more widespread conifers, including western larch, due to subalpine larch's relatively narrow and discontinuous distribution, but it has not been estimated. The relatively high level of population differentiation revealed by microsatellite markers for this species supports this supposition (D. Khasa, Université Laval, pers. comm.). A small but significant deficiency of heterozygotes detected for one allozyme locus compared to Hardy-Weinberg expectations may be indicative of some self-pollination or biparental inbreeding in the species (Semerikov and Lascoux, 1999), but outcrossing rates have not been estimated.

3.2.2. Western larch

Western larch has a mixed mating system, with average estimated multilocus outcrossing rates based on seven allozyme loci of 0.85 (El-Kassaby and Jaquish, 1996). This species has an active pollination mechanism, whereby the female strobilus directs pollen to the nucellus, and this mechanism does not discriminate among self, related or unrelated pollen, which may increase selfing (El-Kassaby and Jaquish, 1996). The relationship between stand density and outcrossing rate is unclear (Fins and Seeb, 1986). The degree of population differentiation for this species is typical of western conifers (Fins and Seeb, 1986; Hamrick *et al*, 1992; Semerikov and Lascoux, 1999), indicating relatively high levels of gene flow.

3.2.3. Tamarack

Tamarack also has a mixed mating system, with a somewhat lower outcrossing rate than most conifers. Using allozyme markers, Knowles *et al*. (1987) estimated the mean multilocus outcrossing rate in five populations as 0.73. Higher stand densities appeared to be related to higher outcrossing estimates. Tamarack likely has fairly high levels of gene flow like most conifers, as indicated by a relatively low degree of population differentation (Cheliak *et al*, 1988), but available methods for indirectly estimating this parameter are poor (Whitlock and McCauley, 1999).

3.3 Seed production

3.3.1. Subalpine larch

Subalpine larch only produces large seed crops about 1 year out of 10, and smaller crops are also relatively rare. Substantial seed is not produced until trees are at least 80 years old, with large, dominant older trees producing the largest crops (Arno, 1990; Arno et al, 1995). Most seeds are released from cones in September. The winged seeds are wind dispersed. There are between 230,000 and 360,500 cleaned seeds per kg (Schopmeyer, 1974). Subalpine larch seed germinates well after a 30-day stratification on a slightly acidic medium or after a treatment with 1% hydrogen peroxide for up to

24 hours (Shearer and Carlson, 1993; Carlson, 1994). Seed collection and handling guidelines for Larix species are available in Schopmeyer (1974).

3.3.2. Western larch

Western larch seed production is usually good, but cone crops vary substantially by year and location. Trees as young at 8 years old can produce seed cones, but they are only start being produced abundantly on trees 40 to 50 years of age. Trees continue to bear large crops for several centuries (Schmidt *et al*, 1976). Long-term records of western larch seed production in Montana show that abundant seed crops are produced at about 5-year intervals. Trees originating from grafted, mature scion produce approximately twice as many cones as those of seedling origin, and five times as many as rooted cuttings (Fins and Reedy, 1995). Cones usually begin to open by early September, but in cool moist summers cone opening may be delayed a month or longer. More than 80% of the seeds usually are dispersed by mid-October. A relatively small proportion of total seed are usually filled, due to a variety of pre- and post-zygotic factors (Owens *et al*, 1994) including lack of pollination (Owens and Molder, 1979b) and frost damage to developing cones (Webber and Ross, 1995). Cones usually fall from the tree during the subsequent winter, but many may stay attached through the next summer. Western larch seeds are relatively small, averaging 200,000 per kg (Schopmeyer, 1974). A cone can contain up to 80 seeds, but on average cones only have half this number. Successfully western larch seed pretreatments include 12 to 24 hours of 3% hydrogen peroxide, or soaking seeds for 18 days at 1°C (Schmidt, 1962; Shearer and Halvorson, 1967).

3.3.3. Tamarack

Tamaracks as young as 5 or 6 years of age can produce both pollen and seed cones (Fowler *et al*, 1995); however, seed production in large quantities does not usually occur until about 75 years of age (Johnston, 1990). Vigorous, open-grown trees 50 to 150 years old produce the best cone crops. In a good year, a single tree may produce up to 20,000 cones with more than 300,000 full seeds. Good seed crops occur at 3 to 6 year intervals, with some seed produced in intervening years. Empty cones remain on trees for 2 to 5 years (Johnston, 1990). The wind-dispersed seeds are approximately 3 mm long, with a 6 mm long chestnut-brown wing. There are between 550,000 and 710,000 cleaned seeds per kg, on average (Schopmeyer, 1974). Tamarack can reproduce well as far as 60 m from the seed-bearing trees if favourable seedbeds are present (Johnston, 1975). Unstratified tamarack seeds germinate well in light at warmer temperatures, but stratified seeds can germinate in the dark at cooler temperatures as well (Farmer and Reinholt, 1986).

3.4. Natural regeneration

3.4.1. Subalpine larch

Subalpine seems to require full light but low temperatures for regeneration (Arno, 1990). It is difficult to regenerate or cultivate even in the relatively cool climates at lower elevations in the Pacific Northwest. Daytime high temperatures and surface drought apparently are lethal. Seed germination is been poor but improves with a 24 hour treatment with a 3% hydrogen peroxide solution (Schopmeyer, 1974). Subalpine larch can have four to six cotyledons, but most individuals have five. All larches have epigeal germination. First-year germinants are seldom found in natural stands. Germination is most successful on northern exposures not fully exposed to afternoon sun on mineral soil. Canopy gaps often contain dense, even-aged cohorts of seedlings or saplings referred to as reproduction glades (Arno, 1990). This typical age distribution suggests that successful reproduction occurs only rarely when conditions are favorable (Arno, 1990). Seedlings grow very slowly above ground the first 20-25 years (Richards, 1981) while seedlings develop extensive root systems while being protected by the snowpack from winter and spring desiccation.

3.4.2. Western larch

Natural regeneration of western larch can be successful provided a reliable seed source, a suitable seedbed, and adequate light are available. Western larch seed disperses up to 240 m from seed trees at the forest margin into open areas (Shearer, 1959). If bare soil is exposed near a seed source, overstocking can result. Dispersal is less uniform in clearcuts than in seed tree and shelterwood silvicultural treatments (Schmidt and Shearer, 1990). Rodent and bird predation reduce seed germination significantly (Stoehr, 2000).

Western larch seeds germinate epigeally about the time of snowmelt, from late April to early June, usually 1 to 2 weeks before associated tree species (Shearer, 1967). Germination is usually rapid and complete after natural stratification during winter. Air temperatures of about 27°C are optimal for germination, but seeds can still germinate at temperatures that are 10° to 15°C cooler (Schopmeyer, 1974).

Western larch is well adapted to mineral soil seedbeds exposed by burning (DeByle, 1981) or mechanical scarification (Schmidt *et al*, 1976; Shearer, 1980). Undisturbed seedbeds of organic matter and areas with heavy root competition have inferior seedling survival. Most mortality occurs during the first growing season; after 3 years seedling losses are minor (Schmidt *et al*, 1976). Seedling survival is primarily impacted by biotic factors early in the growing season and by abiotic factors later. Seedlings established on mineral soil seedbeds are far less susceptible to fungi than those growing on organic substrates. Insolation is the most important abiotic factor impacting seedling survival (Shearer, 1967). Organic substrates result in lethal temperatures earlier and more frequently during the growing season than mineral soils. Drought is the major factor affecting seedling survival later in the growing season, and its effects are greatest in full shade because of competition for moisture by trees and understory vegetation (Schmidt and Shearer, 1990).

Western larch seedlings grow about 5 cm on average the first growing season. Root growth in the first year depends on the conditions. In the shade, roots may average only 2.5 cm the first year, whereas in full light, roots may be over 20 cm long. Subsequent annual height growth averages about 30 cm is typical of the first 4 years (Schopmeyer, 1974; Schmidt and Shearer, 1990).

3.4.3. Tamarack

Tamarack seeds normally germinate between late May and mid-June, and germination peaks at temperatures of 18° to 21°C. Seeds have little or no internal dormancy (Schopmeyer, 1974). Under natural conditions, any existing dormancy is broken during the first winter after seeds are shed (Johnston, 1990). Up to half the seeds that fall may be eaten by rodents, and much of the remaining seed is often damaged by fungi or bacteria. As a result, only 4 to 5% of the seeds may reach germination (Fowells, 1965).

Optimal seedbeds for tamarack are warm, moist, burned mineral or organic soil with no brush but a light herbaceous cover. Slow-growing sphagnum mounds often make a good seedbed, but the moss can provide too much competition under some circumstances. Seedlings in low-light conditions usually grow only 2 to 3 cm the first year and do not usually survive beyond the sixth year; while in full light, they may be as tall as 23 cm the first year and 64 cm the third year. Subsequent growth is generally even more rapid if light is adequate and drainage is good (Fowells, 1965).

3.5. Vegetative reproduction

3.5.1. Subalpine larch

While layering occurs in some larch species and is common in subalpine larch's ecological associate subalpine fir, subalpine larch only rarely spreads by layering (Arno and Habeck, 1972; Arno *et al*, 1995).

Subalpine larch scions have been successfully grafted onto western larch rootstock (B. Jaquish, British Columbia Ministry of Forests, pers. comm.). Given the success of somatic embryogenesis using immature embryo explants in other *Larix* species, it is likely that this technique would be successful for subalpine larch, but it is unlikely to be developed due to the low economic value and lack of need for reforestation with this species. Likewise, methods of organogenesis developed for *Larix gmelinii* (Lin *et al*, 2004) would likely be transferable to all three species of larch discussed here.

3.5.2. Western larch

Western larch does not layer or sprout from stumps or roots in nature. Parent trees are established in seed orchards through grafting (Staubach and Fins, 1988). Juvenile cuttings of western larch root easily, but initially exhibit a high degree of plagiotropism, although most plagiotropic stecklings recover orthotropic growth within 2 years (Edson *et al*, 1995, 1996). Semi-hardwood cuttings collected from July through September yielded higher rooting percentages than softwood cuttings collected in June (Edson *et al*, 1995). Micropropagation methods for multiplying plants using axillary buds have also been developed (Edson *et al*, 1995). Rooted cuttings are not currently used for reforestation due to the poor stock quality. Somatic embryogenesis has been achieved for western larch (Thompson and von Aderkas, 1992; Benkrima and von Aderkas, 1995) but is not being used for operational reforestation. Given the relatively small size of breeding programs for this species, it is unlikely that these programs will adopt a clonal forestry strategy requiring vegetative propagation, but these technologies could be used to overcome seed shortages by bulking seedlots or family seed collections.

3.5.3. Tamarack

Along the northern limit of trees in Canada and Alaska, layering is the dominant type of reproduction for tamarack (Elliott, 1979). Further south, layering is uncommon but may occur when branches are covered by sphagnum moss or drifting sand. Roots are also known to produce adventitious shoots (Fowells, 1965). While tamarack roots easily from juvenile cuttings (Park and Fowler, 1987), the rooting ability of cuttings from 3 to 10 year old donors varied widely among clones, setting dates and donor age (Morgenstern *et al*, 1984). Methods have been developed for somatic embryogenesis in tamarack using excised, immature embryos but these techniques are not yet being used operationally for reforestation. Somatic embryogenesis for *Larix* species provides a system for regeneration following genetic transformation (Klimaszewska *et al*, 1997).

4. Genetics

4.1 Cytology

Subalpine larch, western larch, and tamarack

All species of *Larix*, like most other genera in the Pinaceae, have a haploid number of 12 chromosomes (Wright, 1962). Polyploidy and aneuploidy are rare in conifers. A cross between *L. occidentalis* and *L. decidua* (European larch) produced a single triploid hybrid (Larsen and Westergaard, 1938).

The cytology of reproduction in western larch has been documented by Owens and Molder (1979a, b). The inheritance of plastids appears to be paternal, whereas mitochondrial inheritance is largely but not exclusively maternal, which is typical of other members of the Pinaceae (Neale and Sederoff, 1989; Owens, 1995) and other *Larix* species (DeVerno *et al*, 1993).

4.2. Genetic variation

4.2.1. Population variability

4.2.1.1. Subalpine larch

There are no published estimates of among-population variation in subalpine larch. The isolation and characterisation of 14 microsatellite loci in this species has facilitated ongoing studies of this subject (Khasa *et al*, 2000). Preliminary results of population genetic surveys using these markers indicate much greater population differentiation in subalpine larch than in western larch, as expected given the discontinous distribution of subalpine larch, with estimates of F_{st} of 0.133 for subalpine larch and 0.047 for western larch (D. Khasa, Université Laval, pers. comm.).

4.2.1.2. Western larch

Provenance variation in western larch is significant but clines are considerably flatter than those of some other western conifers with sympatric distributions (Rehfeldt, 1995a, b). Populations separated by 500 m in elevation differ significantly (Rehfeldt, 1995b). Weak but significant clinal variation corresponding to climatic variables, including mean annual temperature and number of frost-free days, has been observed for height growth, phenology, lammas growth, and resistance to *Meria* needle cast (Rehfeldt, 1982, 1992, 1995b). Natural populations in environments differing by 40 frost-free days annually are significantly genetically different, whereas for interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) and Rocky Mountain lodgepole pine (*Pinus contorta* subsp. *latifolia*) in the same region, populations differing by around 20 frost-free days differ significantly (Rehfeldt, 1995a, b). Clinal variation has also been observed for components of shoot growth (Joyce, 1985; Zhang and Fins, 1993).

Population genetics studies of western larch using allozyme analysis have yielded G_{st} estimates ranging from 0.086 to 0.100 (Fins and Seeb, 1986; Semerikov and Lascoux, 1999), indicating that the vast majority of genetic variation is found within rather than among populations. These estimates are similar to the average G_{st} for gymnosperms of 0.073 (Hamrick *et al*, 1992).

4.2.1.3. Tamarack

Provenance testing for this species has not been extensive, probably because of the North American interest in provenances of much faster growing exotic larches such as *L. kaempferi* (Japanese larch), which can grow up to three times as much volume on some sites in eastern Canada (Fowler *et al*, 1988). Tamarack has been infrequently included in the provenance trials of exotic larches, and then only as a control (Boyle *et al*, 1989). Thus, comprehensive provenance trials of tamarack are young relative to those of many other species.

Six-year height growth in a rangewide provenance trial was strongly and negatively correlated with latitude of origin ($r = -0.78$), and moderately correlated with longitude ($r = -0.58$), whereas interaction of provenance by test-site location was significant but weak (Fowler *et al*, 1995). On a smaller geographic scale, Rehfeldt (1970) found significant variation among provenances within Wisconsin (USA) for height, and a positive correlation between date of bud set and parent-tree location frost-free period. Canadian populations in Ontario did not vary significantly for stem form or survival in one study (Boyle *et al*, 1989), but this trial was not designed as a classic provenance trial and included only three populations. In contrast, a study of variation in cold hardiness among 66 Ontario tamarack populations found genetic differentiation over relatively short geographic distances and steep genetic clines, indicating allowable seed transfer distances should be short for this species (Joyce, 1988). In an investigation of the potential to use tamarack as a plantation species on waterlogged sites in France, significant variation was found for growth and form traits among eight provenances from

the southeastern portion of its range. There was an unfavourable correlation between growth rate and stem-form quality (Pâques and Périnot, 1994).

In population genetics studies, the degree of differentiation among populations for allozymes appears to be comparable to that of other conifers, with G_{st} estimated at 0.05 based on 15 loci (Cheliak *et al*, 1988). Although this indicates that most of the genetic variation exists within rather than among populations, in the same study Nei's genetic distance (D) averaged 0.032 among populations, a relatively high estimate for a widespread conifer. On a regional scale, in a study of 44 populations of tamarack from northern Ontario, among-population variation accounted for just 2% of total allozyme variation (Liu and Knowles, 1991). Populations of tamarack in Alaska differ somewhat from those in other areas of the range in cone and needle morphology, supporting the hypothesis of descent from a different Pleistocene refugium than the eastern populations, but the differentiation is not sufficient to warrant recognition of Alaskan tamarack as a separate taxonomic variety (Parker and Dickinson, 1990).

4.2.2. Individual-level variability

4.2.2.1. Subalpine larch

In a comparative allozyme study of both North American and Eurasian larch species, subalpine larch had relatively low levels of heterozygosity in the single population studied (expected heterozygosity of 0.082), lower than all the other species studied except *L. gmelinii* var. *olgensis* (or *L. olgensis*), another larch with a narrow ecological niche (Semerikov and Lascoux, 1999).

4.2.2.2. Western larch

Estimates of expected heterozygosity within populations from allozyme analysis range from 0.082 (Fins and Seeb, 1986) to 0.15 (Semerikov and Lascoux, 1999). The first estimate is somewhat low for a gymnosperm, whereas the second is more typical (Hamrick *et al*, 1992).

Estimated individual heritabilities for growth traits in western larch are higher than for many conifers, averaging 0.25 for height growth (Rehfeldt, 1992). Daily growth rate during the linear portion of the height growth curve has a similar heritability to total height growth but is weakly correlated with shoot phenology, unlike total height growth, and thus may be a better trait for breeding selection (Rehfeldt, 1992).

4.2.2.3. Tamarack

Tamarack has relatively high levels of genetic diversity within stands for both quantitative traits (Rehfeldt, 1970; Jeffers, 1975) and genetic markers. Estimates of expected heterozygosity based on allozyme loci range from 0.10 to 0.22, depending on the populations and allozme loci analyzed (Cheliak *et al*, 1988; Liu and Knowles, 1991; Semerikov and Lascoux, 1999).

Analysis of 16-year height in tamarack progeny trials in Ontario revealed little to no genetic variation among families (Boyle *et al*, 1989), whereas Park and Fowler (1982, 1987) found significant genetic variation for height growth. Narrow-sense heritability for 5-year height over three sites was relatively low, however, with estimates ranging from 0.01 to 0.14 (Park and Fowler, 1987). Clone mean heritabilities are relatively high (0.44 to 0.80), suggesting there may be opportunities to exploit non-additive genetic variation in future breeding programs (Park and Fowler, 1987; Fowler *et al*, 1995).

4.3. Inbreeeding depression and genetic load

4.3.1. Subalpine larch

There are no estimates available of inbreeding depression and genetic load in subalpine larch.

4.3.2. Western larch

There have been no direct studies of inbreeding depression reported for this species. As outcrossing rates for western larch are estimated to be significantly lower than 1, this may indicate that inbreeding depression is less pronounced than in some other conifers (El-Kassaby and Jaquish, 1996).

4.3.3. Tamarack

Tamarack has below-average self-fertility and a high genetic load in terms of lethal equivalents (Fowler *et al*, 1995). Genetically the trees in natural stands are not randomly distributed, and those growing in close proximity are often related (Park and Fowler, 1982). Unlike seeds and seedlings, which have deficiencies of heterozygotes, populations of mature trees approach Hardy-Weinberg expectations for heterozygosity, indicating that there is natural selection against inbred seedlings (Knowles *et al*, 1987; Cheliak *et al*, 1988).

5. Hybridisation

5.1. Subalpine larch and western larch

Subalpine larch and western larch are separated by 400 m or more of elevation in most parts of their ranges. The opportunities for natural hybridisation are limited to the Bitterroot Range in western Montana, where they are sympatric. In this area, *Larix lyallii* hybridises naturally with *L. occidentalis* (Carlson and Blake, 1969; Carlson *et al*, 1990, 1991; Arno *et al*, 1995); however, a usual difference of nearly 2 months in reproductive phenology likely limits their hybridisation (Carlson, 1994). Controlled cross-pollinations between *L. lyallii* females and *L. occidentalis* males result in high seed set, but the reciprocal cross produces few viable seeds (Carlson, 1994). The hybrid offspring are less vigorous than *L. occidentalis* but faster growing than *L. lyallii*, and the stems are (on average) thicker than those of either parent species. Climatic warming may favour these hybrids in sympatric regions. Both subalpine larch and western larch can be artificially hybridised with *L. laricina* (Fowler *et al*, 1995).

A cross between western larch and *L. decidua* (European larch) produced a single hybrid (Larsen and Westergaard, 1938). Western larch can be hybridised with *L. kaempferi* (Japanese larch), and while seed set is low, the seedling offspring can grow approximately twice as fast as intraspecific *L. occidentalis* crosses (Wang, 1971). These interspecific hybrids are not used for operational reforestation in North America (B. Jaquish, British Columbia Ministry of Forests, pers. comm.), and current forest policy on public land discourages use of exotics, including hybrids. Dunkeld larch, *L.* × *marschlinsii* (synonym *L.* × *eurolepis*) (*L. decidua* × *L. kaempferi*), is planted extensively in Europe because of its larch canker resistance (Baltunis and Greenwood, 1998; Mabberley, 1998). In western North America, if either *L. kaempferi* × *L. occidentalis* hybrids or the species *L. kaempferi* were planted on a broad scale, they could have a significant impact on the gene pool of natural western larch populations, given the somewhat weak reproductive barrier between these species and the rapid growth of the hybrids (Wang, 1971). It is not known whether *L. kaempferi* or its hybrids with *L. occidentalis* can also hybridise with subalpine larch. *Larix kaempferi* originates from lower latitudes (in Japan) than North American larches and is less frost hardy than *L. occidentalis* (Wang, 1971); although it can grow as high as 2800 m in its native environment so may have some tolerance for colder environments. The intensive silviculture

necessary for production and establishment of hybrid plantations in North America would likely be directed towards lower elevation, high-productivity sites. If planting of *L. kaempferi* or its hybrids became common, it seems likely that they would be likely be used in areas with substantial elevational separation, thus providing both physical distance and phenological barriers to hybridisation between the plantations and natural populations of subalpine larch.

Attempts at intergeneric *in vitro* hybridisation resulted in western larch pollen germinating and penetrating archegonia of *Pinus monticola* (western white pine). However, successful fertilisation did not occur (Dumont-BéBoux *et al*, 1998).

5.2. Tamarack

Larix laricina has relatively low crossability with other *Larix* species. It has experimentally been crossed most readily with the two other North American larches. Tamarack has also been hybridised with *L. decidua*, *L. sibirica* (Siberian larch), and *L. kaempferi*, but the crossability with these three species was extremely low (Fowler *et al*, 1995). Hybrids between *L. decidua* females with tamarack as the pollen parents were the most vigorous of several *Larix* hybrids evaluated in a trial in Maine (USA) (Baltunis and Greenwood, 1998). Crosses between tamarack and *L. kaempferi* produced little viable seed, but the resulting offspring were relatively vigorous (Baltunis and Greenwood, 1998; Baltunis *et al*, 1998).

6. Ecology

6.1. Climate

6.1.1. Subalpine larch

Subalpine larch is well adapted to a very cold, snowy and generally moist continental subalpine-boreal climate. The extreme lower and upper altitudinal limits of subalpine larch over its entire geographic range are 1520 and 3020 m (Arno, 1990). For more than half of the year, mean temperatures are below freezing. The growing season is defined by mean temperatures above 6°C (Baker, 1944), only lasts about 90 days and is punctuated by occasional frosts and snowfall. July mean temperatures range from approximately 9° to 14°C, but minimum temperatures during the growing season are as low as -5°C and maximums as high as 27°C. January mean temperatures range from -7°C in the northern Cascades to -14°C in Alberta, and long-term record minimum temperatures are likely as low as -50°C near the Continental Divide in Alberta and Montana (Arno, 1990).

Mean annual precipitation for most subalpine larch sites is between 800 and 1,900 mm, the greater amount being more prevalent near the crest of the Cascades. Most stands in Montana's Bitterroot Range receive 1,000 to 1,500 mm. Approximately 75% of this precipitation is snow and sleet. Typically, the snowpack begins to accumulate by late October. By mid-April, it reaches a maximum depth averaging about 2 m in stands near the Continental Divide and about 3 m farther west. The snowpack does not melt away in most stands until early July. The average snowfall is about 10 m in most stands west of the Continental Divide (Arno, 1990).

6.1.2. Western larch

Western larch grows predominantly in continental cool-temperate climates, and marginally in subalpine-boreal climates (Klinka *et al*, 2000). Mean annual temperature within its native range is about 7°C; ranging from an average annual maximum of 29°C to an average annual minimum of -9°C (Schmidt and Shearer, 1990). Average growing season temperatures from May to August are approximately 16°C, with July the warmest month. The frost-free season varies from 60 to 160 days,

usually from early June through early September; however, frosts can occur in any month of the year (Schmidt *et al*, 1976).

Annual precipitation ranges from an average around 710 mm in the northern part of the larch's range to 810 mm in the south, with extremes of 460 mm and 1,270 mm. Snow accounts for over half of the total precipitation on montane and subalpine sites. About one-fifth of the annual precipitation occurs during the growing season, mostly in May and June. July and August are usually dry, with clear sunny days, low humidity, and high evaporation rates (Fowells, 1965).

6.1.3. Tamarack

Tamarack grows under a wide range of climatic conditions across its large range, but predominantly within a montane boreal climate. Across its range, average January temperatures vary from -30° to -1°C, and average July temperatures are 13° to 24°C. The minimum temperatures recorded within the species range vary from -29° to -62°C; the maximum from 29° to 43°C. Tamarack grows with less than a 75 day frost-free period over much of its range, with 120 frost free days in interior Alaska and 180 days along its southern limits. Longer daylength during the growing season generally compensates for the shorter growing season at northern latitudes (Fowells, 1965). Annual precipitation within the range of tamarack is also highly variable, ranging from 180 mm at Fort Yukon, Alaska, to 1,400 mm in eastern Canada. Of this precipitation, 75 to 355 mm is during the growing season. Annual snowfall has a similarly wide variation, from around 100 cm in the District of Mackenzie in northwestern Canada to over five meters near the coasts of Labrador and Quebec (Johnston, 1990).

6.2. Soils

6.2.1. Subalpine larch

The sites occupied by subalpine larch underwent intense glaciation during the Pleistocene and have been deglaciated for less than 12,000 years. As a result, most soils occupied by subalpine larch stands are immature and weakly developed. Short, cool summer temperatures retard chemical weathering. Microbial activity including nitrogen fixation that might enrich the soil is also apparently restricted by low soil temperatures as well as high acidity (Arno, 1990).

Subalpine larch commonly grows on previously unvegetated talus slopes covered with granite or quartzite rock, but the species is absent or scarce on limestone and dolomite (Arno and Habeck, 1972). It can also root in bedrock cracks. This substrate preference is in constrast to several other cold-climate conifers, including *L. sibirica* and tamarack, which often grow on calcium-rich, basic soils (Ritchie, 1957; Hustich, 1966).

Subalpine larch achieves its best growth where soils are kept moist throughout summer by aerated seeps such as in high-elevation basins and near the base of talus slopes. It can also tolerate acidic, organic soils on boggy meadow sites. It is most abundant on cool, north-facing slopes and in high montane basin, but can also grow on south-facing slopes if soils are relatively moist (Arno, 1990).

6.2.2. Western larch

Western larch can tolerate very dry to very moist soil moisture conditions and very poor to very rich soil nutrient conditions, but the most productive growth occurs on fresh to moist, rich to very rich sites. Although this species has a relatively wide climatic and edaphic amplitude, it is infrequent on very moist sites and absent on wet sites. Compared to other tree species, it tolerates water-deficient soils well (Krajina, 1969; Klinka *et al*, 2000).

Relationships between potential site index of western larch and categorical measures of site quality were quantified by New (1999) in the major portion of its British Columbia range. The larch site index

values (1) increased with greater soil water supply from water-deficient to fresh and moist sites, and then decreased with greater water surpluses; and (2) increased from very poor through very rich sites, with the rate of increase diminishing with higher availabilities of nitrogen. The increase in site index values along the soil nutrient gradient was consistently steeper than along the soil moisture gradient (Table 1).

Western larch grows on a wide variety of soils, most commonly on soils that have developed from calcium and magnesium-rich glacial till or colluvium. Most soils supporting the growth of western larch are Inceptisols and Alfisols (Soil Conservation Service, 1975), and infrequently Spodosols which generally occur close to the upper elevation limits of the species (Fowells, 1965).

Table 1. Edatopic grid for western larch showing predicted site index values (m at 50-yr bh), using New's soil moisture and soil nutrient model (n+315, m+/-95% confidence interval), and mean measured site index values according to actual moisture and nutrient regimes

Indicated sample sizes refer only to the predicted and measured site index values

Actual soil moisture regime	Number of plots (n); site index	Actual soil nutrient regime Site index values				
		Very poor	Poor	Medium	Rich	Very rich
Excessively dry	n	3	0	0	0	0
	Predicted	8.6±1.7	9.2	11.5	12.7	13.0
	Measured	8.6	nd	nd	nd	nd
Very dry	n	6	14	8	2	0
	Predicted	13.2±0.6	13.8±0.8	16.1±0.9	17.3±0.7	17.5
	Measured	13.2	14.3	15.4	16.6	nd
Moderately dry	n	0	45	44	6	0
	Predicted	16.7	17.4±0.5	19.6±0.5	20.8±0.5	21.1
	Measured	nd	17.2	19.9	20.8	nd
Slightly dry	n	0	52	58	28	0
	Predicted	19.2	19.9±0.3	22.2±0.5	23.3±0.7	23.6
	Measured	nd	19.9	21.9	23.7	nd
Fresh	n	0	0	18	11	5
	Predicted	20.9	21.5	23.8±0.5	25.0±0.6	25.2±1.1
	Measured	nd	nd	21.9	23.7	25.6
Moist	n	0	0	1	2	5
	Predicted	22.5	23.1	25.4±1.3	26.6±1.3	26.8±1.1
	Measured	nd	nd	26.5	26.2	26.8
Very moist	n	0	0	0	4	1
	Predicted	16.1	16.7	19.0	20.2±1.3	20.4±2.9
	Measured	nd	nd	nd	20.6	18.7
Wet	n	0	0	0	1	0
	Predicted	16.3	17.0	19.2	20.4±1.6	20.7
	Measured	nd	nd	nd	20.4	nd

nd – no data were obtained due to absence or sporadic occurrence of western larch under some edaphic conditions.

6.2.3. Tamarack

Tamarack grows in a wide range of conditions, from moderately dry to wet soil moisture and poor to very rich soil nutrients, although the most productive growth occurs on fresh to moist and nutrient rich to very rich sites (Krajina, 1969; Johnston, 1990; Klinka *et al*, 2000). Compared to many other tree species, it tolerates water-surplus soils well; thus, it grows most commonly on wet organic soils (Histosols - Soil Conservation Service, 1975) developed from sphagnum and woody peats. Woody peat is usually better decomposed, has more nitrogen and mineral nutrients, and is less acidic than sphagnum moss peat. On upland sites the species is associated with mineral soils (especially Inceptisols and Entisols)

that range from coarse sand to heavy clay, and with calcareous soils (Johnston, 1990). Tamarack is more abundant on peatland than upland sites due to its tolerance of high soil moisture, high acidity and low soil temperature. It grows best, however, on moist but well-drained loamy soils in riparian zones and on seep areas, and on mineral soils with a shallow surface layer of organic matter (Fowells, 1965).

6.3. Synecology

6.3.1. Subalpine larch

Subalpine larch is more frequent in pure stands and scattered clumps than in mixed-species stands. It typically forms scattered, open, park-like groves <0.1 ha in size. It is a pioneer species on avalanche slopes, colluvium, and rock outcrops, but can form edaphic climax communities near the upper treeline in association with other subalpine species such as whitebark pine (*Pinus albicaulis*), subalpine fir (*Abies lasiocarpa*), Engelmann spruce (*Picea engelmannii*) and mountain hemlock (*Tsuga mertensiana*) (Eyre, 1980; Klinka *et al*, 2000). Subalpine larch stands are a variant of forest cover type Whitebark Pine (Type 208) but also occur in Engelmann Spruce-Subalpine Fir (Type 206). In British Columbia, subalpine larch is a minor component in the transition between continental high-elevation forest and alpine tundra zones, between 1,800 and 2,300 m (Krajina, 1969; Klinka *et al*, 2000). In the western United States, subalpine larch is a component in the *Tsuga mertensiana* (west of the Cascades) and *Abies lasiocarpa* zones (Franklin and Dyrness, 1973). The understory of most subalpine larch stands throughout the Pacific Northwest is dominated by grouse whortleberry (*Vaccinium scoparium*), smooth woodrush (*Luzula hitchcockii*), mountain arnica (*Arnica latifolia*), and red mountain heather (*Phyllodoce empetriformis*), but on some relatively cold, exposed sites, krumholz subalpine fir and whitebark pine form an understory (Arno, 1970).

6.3.2. Western larch

Western larch may grow in pure stands but is more frequent in mixed-species stands. Old-growth western larch stands are now rare. Despite being a long-lived species, in the absence of fire it is replaced by shade-tolerant species. It is present in all stages of fire-driven, secondary succession. Depending on climate and soil moisture, its most common associates are (1) in the drier temperate climates: interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) and ponderosa pine (*Pinus ponderosa*); (2) in the wetter temperate climates: grand fir (*Abies grandis*), western hemlock (*Tsuga heterophylla*), western redcedar (*Thuja plicata*) and western white pine (*Pinus monticola*); and (3) in subalpine-boreal climates: Engelmann spruce, subalpine fir and mountain hemlock; as well as (4) across its whole range: lodgepole pine (*Pinus contorta* subsp. *latifolia*) (Fowells, 1965; Eyre, 1980; Klinka *et al*, 2000). Western larch stands typically have a diverse understory with dense herbaceous and less dense shrub layers (Pfister *et al*, 1977).

In the United States, western larch comprises the majority of forest cover type Western Larch (212) but is also found in Mountain Hemlock (205), Engelmann Spruce-Subalpine fir (206), Interior Douglas-fir (210), Grand Fir (214), Western White Pine (215), Lodgepole Pine (218), Rocky Mountain Juniper (220), Western Hemlock (224), Western Redcedar-Western Hemlock (227), Western Redcedar (228) and Interior Ponderosa Pine (237) (Schmidt and Shearer, 1990) (Society of American Foresters, 1980). In British Columbia, western larch is a significant component of a number of forest communities in the continental montane forested zones, and to a limited extent in the continental subalpine forest (Krajina, 1969; Klinka *et al*, 2000).

6.3.3. Tamarack

Tamarack forms extensive pure stands in the Canadian boreal forest and in northern Minnesota (USA). In the rest of its range, the species is found locally in both pure and mixed stands. It is a major

component in the forest cover types (Eyre, 1980) Tamarack (Type 38) and Black Spruce-Tamarack (Type 13) and is a minor component in Jack Pine (Type 1), Balsam Fir (Type 5), Black Spruce (Type 12), Red Spruce-Balsam Fir (Type 33), Northern White-Cedar (Type 37), Black Ash-American Elm-Red Maple (Type 39), White Spruce (Type 107), Balsam Poplar (Type 203), Black Spruce (Type 204), Black Spruce-White Spruce (Type 253), and Black Spruce-Paper Birch (Type 254).

Black spruce (*Picea mariana*) is usually found with tamarack in mixed-species stands on all sites. Tamarack stands cast relatively light shade and as a result usually have well-developed shrub, herb, or moss layers featuring a high diversity of species (owing to the extensive range of the species). The moss layer is typically composed of sphagnum (*Sphagnum* spp.) and other bryophytes (Eyre, 1980). Herbs include sedges (*Carex* spp.), cottongrass (*Eriophorum* spp.), false Solomon's seal (*Smilacina trifolia*), marsh cinquefoil (*Potentilla palustris*), marsh-marigold (*Caltha palustris*) and bogbean (*Menyanthes trifoliata*). Shrubs include dwarf and swamp birches (*Betula glandulosa* and *B. pumila*), willows (*Salix* spp.), speckled alder (*Alnus rugosa*), and red-osier dogwood (*Cornus stolonifera*), Labrador-tea (*Ledum groenlandicum*), bog-rosemary (*Andromeda glaucophylla*), leatherleaf (*Chamaedaphne calyculata*) and small cranberry (*Vaccinium oxycoccos*) (Fowells, 1965).

6.4. Stand dynamics

6.4.1. Subalpine larch

Subalpine larch is very shade-intolerant (Arno, 1990). Whitebark pine is also shade-intolerant, but is most abundant on warm-aspect slopes and thus tends to complement rather than compete with the larch (Arno and Habeck, 1972). At the highest elevations and coolest sites, subalpine larch forms parkland-like climax communities owing to its superior hardiness to other subalpine conifers. Its hardiness is due to resistance to the winter desiccation stress that occurs during warm periods when soils are still cold or frozen (Richards, 1981; Richards and Bliss, 1986). Subalpine larch has adaptations to winter dessication stress include deciduous leaves and woody protected buds (Arno, 1970). Its deciduous foliage requires a large amount of moisture throughout the summer, however, compared to evergreens and consequently it occupies relatively moist sites.

Subalpine larch is a long-lived, very slow-growing tree. Height growth is exceedingly slow for the first 20 to 25 years as seedlings become established, but increases rapidly thereafter (Richards, 1981; Richards and Bliss, 1986). Vigorous saplings 1.2 m tall are generally around 30 to 35 years of age. Dominant trees attain small to moderate dimensions, depending upon site conditions, but rare individuals have reached 200 cm in diameter and 30 m in height (Arno, 1990). Leaders grow as short shoots with short internodes in most years, and the annual height increment is substantial in only about 1 in 4 years (Worrall, 1995). Subalpine larch only rarely grows in a shrubby or krummholz form. Although the common life span for dominant trees is 4 to 5 centuries, many individuals attain 700 years, and the oldest trees are estimated to live about 1,000 years (Arno, 1970). Even though subalpine larch frequently forms single-species stands, it can also grow below its usual elevation range in association with subalpine fir, Engelmann spruce and whitebark pine.

6.4.2. Western larch

Western larch is one of the most shade-intolerant conifers in the Pacific Northwest (Klinka *et al*, 2000). Consequently, it grows in even-aged stands. Its primary associates are usually the same age as the larch, but often appear younger due to slower growth. As western larch stands mature, shade-tolerant associates continue to establish and form younger canopy strata (Schmidt and Shearer, 1990). Due to its longevity (often >500 years), western larch is often a persistent seral species, particularly on low-productivity sites.

Fire is essential to the maintenance of western larch in natural populations. High-intensity fires thin stands, reduce fuels, and prepare seedbeds that promote establishment of shade-intolerant conifers, particularly the western larch. Without fire, shade-tolerant associates eventually replace the larch (Schmidt and Shearer, 1990). Even-aged silvicultural systems best fit the ecological requirements of western larch. These systems provide an adequate seed source and the microsite conditions needed for establishment. Site preparation of prescribed burning or scarification to reduce the duff layers and vegetative competition is often necessary for its successful regeneration (Burns, 1983).

6.4.3. Tamarack

Although young seedlings can tolerate some shade, tamarack is very shade-intolerant, and must become dominant to survive, especially in mixed stands (Johnston, 1990). Tamarack is considered a pioneer tree, especially in wetlands. It is generally the first tree to invade filled-lake bogs in primary succession (Fowells, 1965). Tamarack can reproduce successfully on burns (Rowe and Scotter, 1973), so immediately after fire it is one of the early seral tree species on most sites in the boreal forest. Because of its intolerance to shade, tamarack is eventually replaced by black spruce in ombotrophic wetlands, and by northern white-cedar (*Chamaecyparis thyoides*), balsam fir (*Abies balsamea*), and swamp hardwoods in minerotrophic wetlands (Fowells, 1965). Recurring outbreaks of larch sawfly (*Pristiphora erichsonii*) throughout the range of tamarack have probably speeded succession to black spruce or other associates (Eyre, 1980).

In full-light conditions, tamarack is one of the fastest growing conifers on upland sites of the North American boreal forest. On peatlands, tamarack grows faster than any other native conifer. It can reach heights of 24 to 27 m and diameters of 30 to 38 cm. Maximum age is generally 150 to 180 years, but trees 230 to 240 years old are not rare and one individual was documented to have lived to 335 years (Eyre, 1980; Johnston, 1990).

6.5. Damaging agents

6.5.1. Subalpine larch

Violent winds often damage subalpine larch crowns in conjunction with loads of clinging ice or wet snow. If advanced heart rot has so weakened the bole, high winds can break off the trunk causing tree death. The quinine fungus *Fomitopsis officinalis*, which causes brown trunk rot, produces the only conks commonly found on living trunks. Subalpine larch typically suffers little damage from insects or other diseases. Isolated witches' brooms, with dense branch-clusters and branch swelling, are widely scattered in subalpine larch stands, and have been attributed to parasitic dwarf mistletoe (*Arceuthobium laricis*), fungal infection, or perhaps even a genetic abnormality (Arno, 1990).

Avalanches are an important source of damage in high elevation, steep terrain, but the flexibility of younger trees, the strength of trunks larger trees and lack of foliage make subalpine larch less vulnerable to damage than evergreen subalpine trees. Poles up to 13 cm thick and 6 m tall can survive flattening by snowslides, only to straighten again in summer (Arno and Habeck, 1972). Because of their avalanche tolerance, subalpine larch often occupies avalanche paths, forming a disturbance-maintained "disclimax" (Arno, 1990).

6.5.2. Western larch

Mature western larch is a highly fire-resistant tree because of its thick bark, high and open branching habit, and the low flammability of its foliage. Seedlings and saplings have little resistance to fire, but poles are moderately resistant (Fowells, 1965). The species is highly resistant to windthrow because of its extensive root system (Schmidt *et al*, 1976). Immature trees are very sensitive to noxious fumes,

but due to their deciduous foliage, the larches accumulate fewer harmful deposits than evergreen conifers (Carlson and Dewey, 1971).

Dwarf mistletoe (*Arceuthobium laricis*) is the most damaging pathological agent affecting western larch. It can infect seedlings as young as 3 to 7 years old, and infection continues throughout the life of the tree (Wicker and Shaw, 1967). Mistletoe decreases height and diameter growth, kills tree tops, reduces seed viability, creates conditions suitable for other diseases and insects, and causes burls, brashness, and some mortality. Infected residual trees left after harvesting or fire can promptly infect other trees, as mistletoe seed can be ejected as far as 14 m (Smith, 1966). The other important disease found in western larch are needle cast caused by *Hypodermella laricis*, quinine fungus caused by *Fomitopsis officinalis*, and rot caused by *Phellinus pini*. The two most serious insect pests are larch casebearer (*Coleophora laricella*) and western spruce budworm (*Choristoneura occidentalis*) (Schmidt *et al*, 1976).

6.5.3. Tamarack

Tamarack has thin bark and as a result is highly susceptible to fire damage. Its roots are shallow on peatlands, resulting in mortality from all but very light fires. In the boreal forest, tamarack stands have a high surface-fire hazard in the spring but a low crown-fire hazard in pure stands (Rowe and Scotter, 1973). Tamarack stands are often killed by abnormally high water levels. High water levels also result in dieback and the development of adventitious roots and shoots (Denyer and Riley, 1964). Strong winds can uproot large trees growing in swamps or other wet sites where rooting is most shallow. Tamarack is fairly windfirm compared with its common associate black spruce (Johnston, 1990).

The most destructive insect pest of tamarack is the larch sawfly (*Pristiphora erichsonii*). Periodic epidemics of this defoliator occur across Canada and the northern United States. Another serious defoliator of tamarack is the larch casebearer (*Coleophora laricella*). Severe outbreaks have caused extensive mortality of trees of all ages (Johnston, 1990).

Tamarack is host to many pathogens, but none cause sufficient disease to have substantial economic impact. Tamarack is essentially free of stem diseases. The parasitic plant eastern dwarf mistletoe (*Arceuthobium pusillum*) is occasionally found where the tree is growing in mixtures of infected black spruce, but the resulting witches' brooms are small (Hepting, 1971). Several root- and butt-rot fungi reported on tamarack include *Armillaria ostoyae*, *Scytinostroma galactinum*, *Phaeolus schweinitzii*, and *Inonotus tomentosus*. The principal heart-rot fungi are *Fomitopsis officinalis* and *Phellinus pini* (Hepting, 1971). Tamarack is very susceptible to the European larch canker (*Lachnellula willkommii*), but this exotic disease is only a problem in maritime areas in eastern Canada and Maine (USA) (Magasi, 1983).

7. Forestry practices

7.1. Deployment of reforestation materials

7.1.1 Subalpine larch

Subalpine larch is a non-timber species and its wood has essentially no commercial value. As no timber harvesting has been done, even in the best-developed stands, nor does any seem likely on the upper subalpine sites in the future, and considering cultivation difficulties, there has not been any need for seedling production for the species. On an experimental basis, seedlings have been successfully grown and outplanted (Arno *et al*, 1995).

7.1.2. Western larch

Western larch is the fastest-growing and largest of the larches in Pacific Northwest forests, and the most important native timber species of the genus. The presence of this species in pure as well as mixed-species stands is valuable where multiple resource use is the major management objective. Depending on the site and management objective, clearcutting, patch-cutting, strip-shelterwood, and seed-tree systems are suitable for growing western larch (Burns, 1983). Considering its shade intolerance and fast growth rate, western larch is a desirable component in mixed-species stands including shade-intolerant trees, such as ponderosa pine or lodgepole pine (*P. contorta* subsp. *latifolia*), or shade-tolerant trees, such as western redcedar or western hemlock. Propagation by seed is the only contemporary method for regenerating western larch. Techniques for collection, processing, testing, and storage of seed are given in Schopmeyer (1974). Small, infrequent cone crops have resulted in intermittent seed shortages for artificial regeneration (Schmidt and Shearer, 1990). Natural regeneration (where applicable) or planting, using a containerised stock, is used for establishment. Burning or scarification is required for successful natural regeneration of western larch (Schmidt *et al*, 1976; Shearer, 1980; DeByle, 1981).

Genetically improved seed is produced in seed orchards where selected parents are grafted onto conspecific rootstock. In British Columbia, two first-generation seed orchards produce approximately 50% of the total seed currently needed for reforestation for western larch (Forest Genetics Council of British Columbia, 2001). In approximately 5 years, these orchards should provide all of the seed for reforestation in the two major seed planning zones for this species.

7.1.3. Tamarack

Tamarack is a small to medium-sised tree that is a timber species primarily in eastern North America. Its pronounced shade intolerance requires even-aged silvicultural systems, with adaptation of clearcutting or seed-tree cutting. Regeneration often requires some type of site preparation, such as slash disposal or herbicide spraying (Johnston, 1975). Techniques for collection, processing, testing, and storage of seed are given in Schopmeyer (1974). Tamarack stands can be established either through natural regeneration or through planting, of containerised seedlings. Seedling root:shoot ratio must be balanced, seedlings dormant, and a wide spacing used for successful plantation establishment (Johnston, 1990). All the seed currently used for planting in eastern Canada's Maritime region comes from first-generation grafted seed orchards (Fowler *et al*, 1995).

7.2 Provenance transfer

7.2.1. Subalpine larch

Due to the lack of planting of this species, as well as the difficulties in obtaining viable seed, no provenance trials have been established to provide provenance transfer guidelines. In the absence of additional information, for restoration purposes the use of locally collected seed would be advisable. In British Columbia, seed transfer rules for species lacking provenance trial data, including subalpine larch, are 1° latitude S, 2° latitude N, 3° longitude W, 2° longitude E, and 300 m up or 200 m down in elevation from the collection location, based on provenance trial results for other tree species (British Columbia Ministry of Forests, 1995).

7.2.2. Western larch

Local seed zones and breeding programs provide locally adapted seed. In British Columbia, under the Forest Practices Code, western larch seed collected from natural stands can be used for reforestation on sites up to 1° latitude S, 2° latitude N, 3° longitude W, 2° longitude E, and 300 m up or 200 m down

in elevation (British Columbia Ministry of Forests, 1995). In southern British Columbia, there are two local breeding zones for western larch, each with one seed orchard providing improved seed for reforestation in that zone (Forest Genetics Council of British Columbia, 2001). Based on seedling genecological studies, Rehfeldt (1995a, b) concluded that seed should be used within ±225 m of where it is collected. Provenance transfer may offer some gains in growth, but further research on risk of maladaptation is needed to test this hypothesis (Rehfeldt, 1995b).

7.2.3. Tamarack

Based on the limited data available, tamarack shows similar patterns of variation to other widespread conifers, and local seed zones and breeding programs provide locally adapted seed. Local provenances or those from slightly south of local typically appear to be among the best (Jeffers, 1975; Riemenschneider and Jeffers, 1980). In Ontario, seed for all species is managed within 38 seed zones based on a climate model for the province (D. Joyce, Ontario Ministry of Natural Resources, pers. comm.). In British Columbia, seed transfer limits are the same for tamarack as subalpine larch (see above). In Alberta, transfer of seed for all conifers including tamarack is limited to 80 km and 150 m in elevation (N. Dhir, Alberta Forest Service, pers. comm.).

7.3. Breeding programmes

7.3.1. Subalpine larch

There are no breeding programs for subalpine larch, nor are there likely to be, based on the lack of harvesting and artificial reforestation for this species.

7.3.2. Western larch

There are active breeding programs for western larch in both British Columbia and the Inland Northwest region of the United States. Based on the substantial variation within seed and breeding zones for polygenic traits (Joyce, 1985; Fins and Rust, 1989; Rehfeldt, 1992; Zhang and Fins, 1993), potential gains of up to 20% could be achieved in the first generation of selection (Rehfeldt, 1995b). The British Columbia Ministry of Forests breeding program has approximately 600 plus-trees in progeny tests, and grafted seed orchards will be rogued based on the results from these tests, primarily evaluating for growth rate, with wood density as a secondary trait (Forest Genetics Council of British Columbia, 2001). A similar breeding program is underway by the Inland Empire Tree Improvement Cooperative, based at the University of Idaho.

7.3.3. Tamarack

There are active breeding programs for tamarack in Quebec and the Maritime provinces in Canada, although planting is not extensive (Fowler *et al*, 1995). These breeding programs are approaching the second generation. Parent trees are evaluated for general combining ability based on the performance of progeny from polycrosses. Grafted clonal seed orchards are rogued based on the results of these progeny trials. Ontario no longer has an active breeding program for tamarack (D. Joyce, Ontario Ministry of Natural Resources, pers. comm.). In the Maritime region, there are approximately 300 plustrees under evaluation in progeny tests (Fowler *et al*, 1995). First generation clonal seed orchards containing grafted ramets of these parents will be rogued based on the results of these tests for growth and form traits. High clonal heritabilities for economic traits in this species indicate an opportunity to exploit non-additive genetic variation in future breeding programs (Fowler *et al*, 1995).

7.4. Conservation of genetic resources

7.4.1. Subalpine larch

As subalpine larch is neither harvested nor planted, and occurs in high-elevation ecosystems that are relatively well represented in natural parks, wilderness areas, and other conservation areas, gene conservation for this species is accomplished through *in situ* protection in established reserves. In British Columbia, subalpine larch was found to be well protected in existing reserves (Lester and Yanchuk, 1996). The greatest threat to subalpine larch and other high-elevation species in terms of genetic resources is climate change, as the rate of climate change may exceed the maximum migration rate of species, and high-elevation species exist in discontinuous ecosystems (Aitken, 2000).

7.4.2. Western larch

Western larch genetic resources are being maintained both *in situ* in established protected areas such as natural parks and ecological reserves, and *ex situ* in seed and clone banks, breeding arboreta and genetic field tests. A survey of the degree of protection of conifer genetic resources in British Columbia in 1996 concluded that western larch was adequately protected at that time (Lester and Yanchuk, 1996), and reserves have nearly doubled in area since then. Rehfeldt (1995b) suggested that while the controlled, local collection and deployment of seed and localised breeding programs for this species protect the natural genetic structure and diversity, it may be prudent to establish gene pool reserves for western larch in some areas.

7.4.3. Tamarack

Given the broad distribution of tamarack, its presence in many unharvested ecosystems such as bogs, and the use of natural regeneration or local provenances as seed sources for planting, the genetic resources of tamarack likely are being well conserved. A thorough gap-analysis only of the *in situ* protected status of this species for the small portion of its range within British Columbia has been published (Lester and Yanchuk, 1996).

8. Summary

***Subalpine larch**:*

The primary values of subalpine larch are for watershed protection, wildlife habitat, outdoor recreation, and aesthetics. The ability to occupy steep north slopes and snow chutes where other trees can scarcely grow suggests that it helps to stabilise snow loads and reduce the severity of avalanches. The unusual hardiness of this species and its adaptations for survival on environmentally extreme sites make it of special interest for scientific study, and reclamation plantings on subalpine sites. There is a tremendous lack of genetic information on this species, and genetic management is limited to in situ gene conservation. The current degree of protection of genetic resources of subalpine larch in natural parks is adequate. Climate change is the primary threat to this high-elevation species.

***Western larch**:*

Western larch, a species with relatively narrow ecological amplitude, is one of the important and valuable timber crop species in western North America. Across its range it functions predominantly as a long-lived seral and fire-adapted species. Because of its rapid growth rate, western larch produces a higher volume of wood sooner than many of its associates. It is an exposure-requiring species that is easy to regenerate. Although growing typically in even-aged stands, it may associate even in early stages of secondary succession with several shade-tolerant tree species forming stratified uneven-age stands, and be a prominent component in many ecosystems in several climatic zones in the Pacific Northwest. Western larch is not only an important timber species but also a major tree cover in many scenic and recreational areas and critical watersheds. The seasonal change in hue of foliage from light green in the spring and summer, to gold in the fall, enhances the beauty of these montane forests. The genetic base of this species is well-protected in existing natural parks and reserves over most of its range. Despite available technologies for somatic embryogenesis and genetic transformation, and its rapid growth rate and high wood quality, genetic improvement is limited to local selective breeding due to the relatively small numbers of seedlings planted annually, primarily on public lands within its native range. Genetic transformation and tissue culture methods developed for other Larix species could likely be adapted for western larch.

***Tamarack**:*

Tamarack is a major component of the North American boreal forest, with a very wide ecological amplitude. Across its extensive range, tamarack functions predominantly as a pioneer and early seral, relatively short-lived, and fire-adapted species. Local, small breeding programs have been established and will likely continue for this species in the eastern portion of the range. While somatic embryogenesis and genetic transformation technologies are available, it is unlikely these or other biotechnological methods will be applied on a large scale due to the slow growth rates, long rotations and relatively few seedlings planted annually for this species. Genetic transformation and somatic embryogenesis techniques have been developed for tamarack.

References

Aitken, S.N. 2000. Conserving adaptive variation in forest ecosystems. J. Sustain. For. 10: 1-12.

Arno, S.F. 1970. Ecology of alpine larch (*Larix lyallii* Parl.) in the Pacific Northwest. Ph.D. thesis, University of Montana, Missoula, Montana. 264 pp.

Arno, S.F. 1990. *Larix lyallii* Parl. – Alpine larch. Pp. 152-159 *in* R.M. Burns and B.H. Honkala (technical coordinators), Silvics of North America, Vol. 1. USDA Agriculture Handbook 654. Washington, D.C.

Arno, S.F. and J.R. Habeck. 1972. Ecology of alpine larch (*Larix lyallii* Parl.) in the Pacific Northwest. Ecol. Monogr. 42: 417-450.

Arno, S.F., J. Worrall and C.E. Carlson. 1995. *Larix lyallii*: Colonist of tree-line and talus sites. Pp. 72-78 *in* W.C. Schmidt and K.J. McDonald (compilers), Ecology and management of *Larix* forests: A look ahead. Proceedings of an international symposium, 5-9 Oct. 1992, Whitefish, Montana, USA.

Baker, F.S. 1944. Mountain climates of the western United States. Ecol. Monogr. 14: 223-254.

Baltunis, B.S. and M.S. Greenwood. 1998. Variation in lateral shoot elongation patterns and hybrid vigor in full-sib families and interspecific hybrids of larch. Tree Physiol. 19: 131-136.

Baltunis, B.S., M.S. Greenwood and T. Eysteinsson. 1998. Hybrid vigor in *Larix*: Growth of intra- and interspecific hybrids of *Larix decidua*, *L. laricina*, and *L. kaempferi* after 5 years. Silvae Genet. 17: 288-293.

Benkrima, L. and P. von Aderkas. 1995. *In vitro* embryogenesis in larch. Pp. 412-416 *in* W.C. Schmidt and K.J. McDonald (compilers), Ecology and management of *Larix* forests: A look ahead. Proceedings of an international symposium, 5-9 Oct. 1992, Whitefish, Montana.

Boyle, T.J.B., T.C. Nieman, S. Magnussen and J. Veen. 1989. Species, provenance, and progeny tests of the genus *Larix* by the Petawawa National Forestry Institute. Petawawa National Forestry Institute Information Report PI-X-94. 70 pp.

British Columbia Ministry of Forests. 1995. Seed and vegetative material guidebook. Forest Practices Code of British Columbia. Victoria, B.C. 57 pp.

Burns, R.M. (technical compiler). 1983. Silvicultural systems for the major forest types of the United States. USDA Agriculture Handbook 445. Washington, D.C. 191 pp.

Carlson, C.E. 1994. Germination and early growth of western larch (*Larix occidentalis*), alpine larch (*Larix lyallii*), and their reciprocal hybrids. Can. J. For. Res. 24: 911-916.

Carlson, C.E. and G.M. Blake. 1969. Hybridization of western and subalpine larch. Montana Forest and Conservation Experiment Station Bulletin 37, School of Forestry, University of Montana, Missoula. 12 pp.

Carlson, C.E. and G.E. Dewey. 1971. Environmental pollution by fluorides in Flathead National Forest and Glacier National Park. USDA Forest Service, Division of State and Private Forestry, Forest Insect and Disease Branch, Missoula, Montana. 55 pp.

Carlson, C.E., S.F. Arno and J. Menakis. 1990. Hybrid larch of the Carlton Ridge Research Natural Area in western Montana. Nat. Areas J. 10: 134-139.

Carlson, C.E., R.G. Gates and S.G. Spencer. 1991. Foliar terpenes of a putative hybrid swarm (*Larix occidentalis* Nutt. × *L. lyallii* Parl.) in western Montana. Can. J. For. Res. 21: 876-881.

Cheliak, W.M., J. Wang and J.A. Pitel. 1988. Population structure and genic diversity in tamarack, *Larix laricina* (Du Roi) K. Koch. Can. J. For. Res. 18: 1318-1324.

DeByle, N.V. 1981. Clearcutting and fire in the larch/Douglas-fir forests of western Montana – a multifaceted research summary. USDA Forest Service, General Technical Report INT-99. Intermountain Forest and Range Experiment Station, Ogden, Utah. 73 pp.

Denyer, W.B.G. and C.G. Riley. 1964. Dieback and mortality of tamarack caused by high water. For. Chron. 40: 334-338.

DeVerno, L.L, P.J. Charest and L. Bonen. 1993. Inheritance of mitochondrial DNA in the conifer *Larix*. Theor. Appl. Genet. 86: 383-388.

Dumont-BéBoux, N., B.R. Anholt and P. von Aderkas. 2000. *In vitro* germination of western larch pollen. Can. J. For. Res. 30: 329-332.

Dumont-BéBoux, N., M. Weber, Y. Ma and P. von Aderkas. 1998. Intergeneric pollen-megagametophyte relationships of conifers *in vitro*. Theor. Appl. Genet. 97: 881-887.

Edson, J.L., D.L. Wenny and L. Fins. 1995. Vegetative propagation of western larch. Pp. 197-208 *in* W.C. Schmidt and K.J. McDonald (compilers), Ecology and management of *Larix* forests: A look ahead. Proceedings of an international symposium, 5-9 Oct. 1992, Whitefish, Montana.

Edson, J.L., D.L. Wenny, L. Fins and L.W. Roberts. 1996. Growth and form of western larch stecklings: Plagiotropism and reiteration. Can. J. For. Res. 26: 1273-1283.

El-Kassaby, Y.A. and B. Jaquish. 1996. Population density and mating pattern in western larch. J. Heredity 87: 438-443.

Elliott, D.L. 1979. The current regenerative capacity of the northern Canadian trees, Keewatin, N.W.T., Canada: Some preliminary observations. Arct. Alp. Res**.** 11: 243-251.

Eyre, F.H. (ed.). 1980. Forest types of the United States and Canada. Society of American Foresters, Washington, D.C. 148 pp.

Eysteinsson, T. and M.S. Greenwood. 1995. Flowering on long and short shoots of *Larix laricina* in response to differential timing of $GA_{4/7}$ applications. Tree Physiol. 15: 467-469.

Farmer, R.E. and R.W. Reinholt. 1986. Seed quality and germination characteristics of tamarack in northwestern Ontario. Can. J. For. Res. 16: 680-683.

Fins, L. and V. Reedy. 1995. Comparison of cone production by rooted cuttings, grafts and seedling origin trees of western larch. Pp. 422-424 *in* W.C. Schmidt and K.J. McDonald (compilers), Ecology and management of *Larix* forests: A look ahead. Proceedings of an international symposium, 5-9 Oct. 1992, Whitefish, Montana.

Fins, L. and M. Rust. 1989. Heritability and genetic gain in western larch. Pp. 46-51 *in* L. Fins (ed.), Inland Empire Tree Improvement Cooperative, 13th Progress Report. University of Idaho, Forest, Wildlife and Range Experiment Station, Moscow, Idaho.

Fins, L. and L.W. Seeb. 1986. Genetic variation in allozymes of western larch. Can. J. For. Res. 16: 1013-1018.

Forest Genetics Council of British Columbia. 2001 (July). Business Plan 2001/2002. Victoria, B.C. 19 pp. plus appendices.

Fowells, H.A. (compiler). 1965. Silvics of forest trees of the United States. USDA Agriculture Handbook 271. Washington, D.C. 762 pp.

Fowler, D.P., J.D. Simpson, Y.S. Park and M.H. Schneider. 1988. Yield and wood properties of 25-year-old Japanese larch of different provenances in eastern Canada. For. Chron. 64: 475-479.

Fowler, D.P., Y.S. Park and J. Loo-Dinkins. 1995. *Larix laricina* – silvics and genetics. Pp. 54-57 *in* W.C. Schmidt and K.J. McDonald (compilers), Ecology and management of *Larix* forests: A look ahead. Proceedings of an international symposium, 5-9 Oct. 1992, Whitefish, Montana.

Franklin, J.F. and C.T. Dyrness. 1973. Natural vegetation of Oregon and Washington. USDA Forest Service, General Technical Report PNW-8, Pacific Northwest Forest and Range Experimental Station, Portland, Oregon. 416 pp.

Gernandt, D.S. and A. Liston. 1999. Internal transcribed spacer region evolution in *Larix* and *Pseudotsuga*. Am. J. Bot. 86: 711-723.

Hamrick, J.L., M.J.W. Godt and S.L. Sherman-Broyles. 1992. Factors influencing levels of genetic diversity in woody plant species. New For. 6: 95-124.

Hepting, G.H. 1971. Diseases of forest and shade trees of the United States. USDA Agriculture Handbook 386. Washington, D.C. 658 pp.

Hosie, R.C. 1979. Native trees of Canada, 8th Ed. Fitzhenry and Whiteside Ltd., Don Mills, Ont. 380 pp.

Hustich, I. 1966. On the forest-tundra and the northern tree-lines. Report from Kevo Subarctic Research Station 3. SARJA-Series A. Biologica Geographica 36: 7-47.

Jeffers, R.M. 1975. Survival and height growth of tamarack planted in northern Wisconsin. USDA Forest Service Research Note NS-190.

Johnston, W.F. 1975. Reproducing lowland conifer forests. J. For. 73: 17-20.

Johnston, W.F. 1990. *Larix laricina* (Du Roi) K. Koch – tamarack. Pp. 141-151 *in* R.M. Burns and B.H. Honkala (technical coordinators), Silvics of North America, Vol. 1. USDA Agriculture Handbook 654. Washington.

Joyce, D. 1985. Juvenile shoot growth and yield potential in western larch. Ph.D. dissertation, University of Idaho, Moscow, Idaho. 159 pp.

Joyce, D. 1988. Adaptive variation in cold hardiness of eastern larch, *Larix laricina*, in northern Ontario. Can. J. For. Res. 18: 85-89.

Khasa, P.D., C.H. Newton, M.H. Rahman, B. Jaquish and B.P. Dancik. 2000. Isolation, characterization and inheritance of microsatellite loci in alpine larch and western larch. Genome 43: 439-448.

Klimaszewska, K., Y. Devantier, D. Lachance, M.A. Lelu and P.J. Charest. 1997. *Larix laricina* (tamarack): Somatic embryogenesis and genetic transformation. Can. J. For. Res. 27: 538-550.

Klinka, K., J. Worrall, L. Skoda and P. Varga. 2000. The distribution and synopsis of ecological and silvical characteristics of tree species of British Columbia's forests. Can. Cartographics Ltd., Coquitlam, B.C. 180 pp.

Knowles, P., G.R. Furnier, M.A. Aleksiuk and D.J. Perry. 1987. Significant levels of self-fertilization in natural populations of tamarack. Can. J. Bot. 65: 1087-1091.

Krajina, V.J. 1969. Ecology of forest trees in British Columbia. Ecol. West. North Am. 2: 1-146.

Krüssmann, G. 1985. Manual of cultivated conifers. Timber Press, Portland, Oregon. 361 pp.

Larsen, C.S. and M. Westergaard. 1938. Contributions to the cytogenetics of forest trees. I. A triploid hybrid between *Larix decidua* and *L. occidentalis*. J. Genet. 36: 523-530.

Lester, D.T. and A.D. Yanchuk. 1996. A survey of the protected status of conifers in British Columbia: *In situ* gene conservation. Province of British Columbia Ministry of Forests Research Program Research Report 04. 34 pp.

Lin, X.F., Zhang W.G., Takano H., Tako S., Ono K. 2004. Efficient plant regeneration and micropropagation from callus derived from mature zygotic embryos of *Larix gmelinii*. Plant Biotechnol. 21: 159-163.

Little Jr., E.L. 1979. Checklist of United States trees. USDA Forest Service, Agriculture Handbook No. 541. Washington, D.C. 375 pp.

Liu, Z. and P. Knowles. 1991. Patterns of allozyme variation in tamarack (*Larix laricina*) from northern Ontario. Can. J. Bot. 69: 2468-2474.

Mabberley, D.J. 1998. The Plant-Book: A Portable Dictionary of the Higher Plants, 2nd Ed., revised printing. Cambridge University Press, Cambridge, England, U.K. 858 pp.

Magasi, L.P. 1983. Forest pest conditions in the Maritimes 1982. Canadian Forestry Service Maritime Forest Research Centre, Info. Rept. M-X-141. 41 pp.

Morgenstern, E.K., J.M. Nicholson and Y.S. Park. 1984. Clonal selection in *Larix laricina*. I. Effects of age, clone and season on rooting of cuttings. Silvae Genet. 33: 155-160.

Neale, D.B. and R.R. Sederoff. 1989. Paternal inheritance of chloroplast DNA and maternal inheritance of mitochondrial DNA in loblolly pine. Theor. Appl. Genet. 77: 212-216.

New, D.M. 1999. Productivity of western larch in relation to categorical measures of climate, soil moisture, and soil nutrients. M.Sc. thesis, University of British Columbia, Vancouver, B.C. 75 pp.

Owens, J.N. 1995. Reproductive biology of larch. Pp. 97-109 *in* W.C. Schmidt and K.J. McDonald (compilers), Ecology and management of *Larix* forests: A look ahead. Proceedings of an international symposium, 5-9 Oct. 1992, Whitefish, Montana.

Owens, J.N. and M. Molder. 1979a. Bud development in *Larix occidentalis*. II. Cone differentiation and early development. Can. J. Bot. 57: 1557-1572.

Owens, J.N. and M. Molder. 1979b. Sexual reproduction of *Larix occidentalis.* Can. J. Bot. 57: 2673-2690.

Owens, J.N., S.J. Morris and G.L. Catalano. 1994. How the pollination mechanism and prezygotic and postzygotic events affect seed production in *Larix occidentalis*. Can. J. For. Res. 24: 917-927.

Pâques, L.E. and C. Périnot. 1994. Performance à 25 ans de quelques provenances de mélèze laricin (*Larix laricina* (Du Roi) Koch) sur sols hydromorphes. Ann. Sci. For. 51: 357-372.

Park, Y.S. and D.P. Fowler. 1982. Effects of inbreeding and genetic variances in a natural population of tamarack (*Larix laricina* [Du Roi] K. Koch) in eastern Canada. Silvae Genet. 31: 21-26.

Park, Y.S. and D.P. Fowler. 1987. Genetic variances among clonally propagated populations of tamarack and the implications for clonal forestry. Can. J. For. Res. 17: 1175-1180.

Parker, W.H. 1993. *Larix* Miller. Pp. 366-368 *in* Flora of North America Editorial Committee (ed.), Flora of North America North of Mexico, Vol. 2: Pteridophytes and Gymnosperms. Oxford University Press, New York.

Parker, W.H. and T.A. Dickinson. 1990. Range-wide morphological and anatomical variation in *Larix laricina*. Can. J. Bot. 68: 832-840.

Pfister, R.D., B. Kovalchik, S. Arno and R. Presby. 1977. Forest habitat types of Montana. USDA Forest Service, General Technical Report INT-34, Intermountain Forest and Range Experiment Station, Ogden, Utah. 174 pp.

Qian, T., R.A. Ennos and T. Helgason. 1995. Genetic relationships among larch species based on analysis of restriction fragment variation for chloroplast DNA. Can. J. For. Res. 25: 1197-1202.

Rehfeldt, G.E. 1970. Genecology of *Larix laricina* (Du Roi) K. Koch in Wisconsin. I. Patterns of natural variation. Silvae Genet. 19: 9-16.

Rehfeldt, G.E. 1982. Differentiation of *Larix occidentalis* populations from the Northern Rocky Mountains. Silvae Genet. 31: 13-19.

Rehfeldt, G.E. 1992. Breeding strategies for *Larix occidentalis*: adaptations to the biotic and abiotic environment in relation to improving growth. Can. J. For. Res. 22: 5-13.

Rehfeldt, G.E. 1995a. Domestication and conservation of genetic variability in western larch. Pp. 91-96 *in* W.C. Schmidt and K.J. McDonald (compilers), Ecology and management of *Larix* forests: A look ahead. Proceedings of an international symposium, 5-9 Oct. 1992, Whitefish, Montana.

Rehfeldt, G.E. 1995b. Genetic variation, climate models and the ecological genetics of *Larix occidentalis*. For. Ecol. Manag. 78: 21-37.

Reimenschneider, D.E. and R.M. Jeffers. 1980. Height and diameter of tamarack seed sources in northern Wisconsin. USDA Forest Serv. Res. Paper NC-190. 6 pp.

Richards, J.H. 1981. Ecophysiology of a deciduous timberline tree, *Larix lyallii* Parl. Ph.D. dissertation, University of Alberta, Edmonton, Alberta. 228 pp.

Richards, J.H. and L.C. Bliss. 1986. Winter water relations of a deciduous timberline conifer, *Larix lyallii* Parl. Oecologia 69: 16-24.

Ritchie, J.C. 1957. The vegetation of northern Manitoba. Ecology 38: 429-435.

Roe, A.L. 1966. A procedure for forecasting western larch seed crops. USDA Forest Service, Research Note INT-49, Intermountain Forest and Range Experiment Station, Ogden, Utah. 7 pp.

Ross, S.D. 1991. Promotion of flowering in western larch by girdling and gibberellin $A_{4/7}$ and recommendations for selection and treatment of seed trees. British Columbia Min. of Forests Research Note 105, Victoria, B.C. 13 pp.

Rowe, J.S. and G.W. Scotter. 1973. Fire in the boreal forest. Quat. Res. 3: 444-464.

Schmidt, W.C. 1962. Rapid viability tests for western larch seed. Montana Academy of Science, Proceedings 21:26-32.

Schmidt, W.C. 1995. Around the world with *Larix*: An introduction. Pp. 6-18 *in* W.C. Schmidt and K.J. McDonald (compilers), Ecology and management of *Larix* forests: A look ahead. Proceedings of an international symposium, 5-9 Oct. 1992, Whitefish, Montana.

Schmidt, W.C. and J.E. Lotan. 1980. Phenology of common forest flora of the Northern Rockies – 1928 to 1937. USDA Forest Service, Research Paper INT-259, Intermountain Forest and Range Experiment Station, Ogden, Utah. 20 pp.

Schmidt, W.C. and R.C. Shearer. 1990. *Larix occidentalis* Nutt. – western larch. Pp. 160-172 *in* R.M. Burns and B.H. Honkala (technical coordinators), Silvics of North America, Vol. 1. USDA Agriculture Handbook 654. Washington, D.C.

Schmidt, W.C., R.C. Shearer and A.L. Roe. 1976. Ecology and silviculture of western larch forests. USDA Technical Bulletin 1520. Washington, D.C. 96 pp.

Schopmeyer, C.S. (technical coordinator). 1974. Seeds of woody plants in the United States. USDA Agriculture Handbook 450. Washington, D.C. 883 pp.

Semerikov, V.L. and M. Lascoux. 1999. Genetic relationships among Eurasian and American *Larix* species based on allozymes. Heredity 83: 62-70.

Semerikov, V.L. and M. Lascoux. 2003. Nuclear and cytoplasmic variation within and between Eurasian *Larix* (Pinaceae) species. Am. J. Bot. 90: 1113-1123.

Semerikov, V.L., L.F. Semerikov and M. Lascoux. 1999. Intra- and interspecific allozyme variability in Eurasian *Larix* Mill. species. Heredity 82: 193–204.

Semerikov, V.L., H. Zhang, M. Sun and M. Lascoux. 2003. Conflicting phylogenies of *Larix (*Pinaceae) based on cytoplasmic and nuclear DNA. Mol. Phylogenet. Evol. 27: 173-184.

Shearer, R.C. 1959. Western larch seed dispersal over clear-cut blocks in northwestern Montana. Montana Academy of Science, Proceedings. 19: 130-134.

Shearer, R.C. 1967. Insolation limits establishment of western larch seedlings. USDA Forest Service, Research Note INT-64. Intermountain Forest and Range Experiment Station, Ogden, Utah. 8 pp.

Shearer, R.C. 1980. Regeneration establishment in response to harvesting and residue management in a western larch-Douglas-fir forest. Pp. 249-269 *in* Proceedings of the Symposium on Environmental Consequences of Timber Harvesting in Rocky Mountain Coniferous Forests. USDA Forest Service, General Technical Report INT-90, Intermountain Forest and Range Experiment Station, Ogden, Utah.

Shearer, R.C. and C.E. Carlson. 1993. Barriers to germination of *Larix occidentalis* and *Larix lyallii* seeds. Pp. 127-132 *in* Edwards DGW, (ed.) Dormancy and barriers to germination. Proceedings. International Symposium of IUFRO Project Group P2.04-00 (Seed Problems): 1991 April 23-26; Victoria, BC: Forestry Canada, Pacific Forestry Centre.

Shearer, R.C. and C.H. Halvorson. 1967. Establishment of western larch by spring spot seeding. J. For. 65:188-193.

Shearer, R.C., M.U. Stoehr, J.E. Webber and S.D. Ross. 1999. Seed cone production enhanced by injecting 38-year-old *Larix occidentalis* Nutt. with $GA_{4/7}$. New For. 18: 289-300.

Smith, R.B. 1966. Hemlock and larch dwarf mistletoe seed dispersal. For. Chron. 42: 395-401.

Society of American Foresters. 1980. Forest cover types in the United States and Canada. F. H. Eyre, ed. Washington, DC. 148 pp.

Soil Conservation Service. 1975. Soil taxonomy (a basic system of soil classification for making interpreting soil surveys). USDA Agriculture Handbook 436. Washington, D.C. 754 pp.

Staubach, M.C. and L. Fins. 1988. Grafting western larch. West. J. Appl. For. 3: 55-56.

Stipanicic, A. 1975. L'amélioration du genre mélèze (*Larix* spp.) au service de la recherche du Ministère des Terres et Forêts du Québec, Mémoire No. 20. 37 pp.

Stoehr, M.U. 2000. Seed production of western larch in seed-tree systems in the southern interior of British Columbia. For. Ecol. Manag. 130: 7-15.

Thompson, R.G. and P. von Aderkas. 1992. Somatic embryogenesis and plant regeneration from immature embryos of western larch. Plant Cell Rep. 11: 379-385.

Vidacovic, M. 1991. Conifers: Morphology and variation. Graficki Zavod Hrvatske, Zagreb, Croatia. Cited in Qian *et al*, 1995.

Viereck, L.A. and E.L. Little Jr.. 1972. Alaska trees and shrubs. USDA Forest Service Agricultural Handbook No. 410. Washington, D.C. 265 pp.

Wang, C.W. 1971. The early growth of *Larix occidentalis* × *L. leptolepis* hybrids. University of Idaho College of Forestry, Wildlife and Range Sciences Station Note No. 17. 4 pp.

Webber, J.E. and S.D. Ross. 1995. Flower induction and pollen viability for western larch. Pp. 395-402 *in* W.C. Schmidt and K.J. McDonald (compilers), Ecology and management of *Larix* forests: A look ahead. Proceedings of an international symposium, 5-9 Oct. 1992, Whitefish, Montana.

Whitlock, M.C. and D.E. McCauley.1999. Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm+1)$. Heredity 82: 117-125.

Wicker, E.F. and G. Shaw. 1967. Target area as a klendusic factor in dwarf mistletoe infections. Phytopathology 57: 1161-1163.

Worrall, J. 1995. Intermittent long and short-shoot growth in subalpine larch leaders. Pp. 282-284 *in* W.C. Schmidt and K.J. McDonald (compilers), Ecology and management of *Larix* forests: A look ahead. Proceedings of an international symposium, 5-9 Oct. 1992, Whitefish, Montana.

Wright, J.W. 1962. Genetics of forest tree improvement. Food and Agriculture Organization of the United Nations, Rome. 399 pp.

Zhang, J.-W. and L. Fins. 1993. Variation in shoot growth components among western larch families. Can. J. For. Res. 23: 1520-1527.

Section 4.
Douglas-Fir (*Pseudotsuga menziesii*)

1. Taxonomy

Pseudotsuga menziesii (Mirbel) Franco is generally called Douglas-fir (so spelled to maintain its distinction from true firs, the genus *Abies*). *Pseudotsuga* Carrière is in the kingdom Plantae, division Pinophyta (traditionally Coniferophyta), class Pinopsida, order Pinales (conifers), and family Pinaceae. The genus *Pseudotsuga* is most closely related to *Larix* (larches), as indicated in particular by cone morphology and nuclear, mitochondrial and chloroplast DNA phylogenies (Silen 1978; Wang *et al*. 2000); both genera also have non-saccate pollen (Owens *et al*. 1981, 1994). Based on a molecular clock analysis, *Larix* and *Pseudotsuga* are estimated to have diverged more than 65 million years ago in the Late Cretaceous to Paleocene (Wang *et al*. 2000). The earliest known fossil of *Pseudotsuga* dates from 32 Mya in the Early Oligocene (Schorn and Thompson 1998).

Pseudostuga is generally considered to comprise two species native to North America, the widespread *Pseudostuga menziesii* and the southwestern California endemic *P. macrocarpa* (Vasey) Mayr (bigcone Douglas-fir), and in eastern Asia comprises three or fewer endemic species in China (Fu *et al*. 1999) and another in Japan. The taxonomy within the genus is not yet settled, and more species have been described (Farjon 1990). All reported taxa except *P. menziesii* have a karyotype of $2n = 24$, the usual diploid number of chromosomes in Pinaceae, whereas the *P. menziesii* karyotype is unique with $2n = 26$. The two North American species are vegetatively rather similar, but differ markedly in the size of their seeds and seed cones, the latter 4-10 cm long for *P. menziesii* and 9-20 cm for *P. macrocarpa* (Elias 1980; Lipscomb 1993). Although additional species have been described that may occur in Mexico (Flous 1934a, 1934b; Martínez 1963) — *P. flahaultii* Flous, *P. guinieri* Flous, *P. lindleyana* (Roezl) Carr., *P. macrolepis* Flous, *P. rehderi* Flous, these taxa are not broadly recognized due to their overlapping morphological characters which do not correlate with the scattered distribution of Mexican populations of the genus (Farjon 1990; Debreczy and Rácz 1995; Reyes-Hernández *et al*. 2005). Considerable morphological variation has been found among 19 populations sampled from the three major geographical regions of Mexico where *Pseudotsuga* occurs (Reyes-Hernández *et al*. 2005). Strong genetic differentiation for isozymes was found between a northeastern Mexican population of *P. menziesii* at approximately 25º N (2500 m elevation) and 103 other populations of the species sampled rangewide (Li and Adams 1989).

Two botanical varieties of Douglas-fir are commonly recognized: *P. menziesii* var. *menziesii*, called coastal Douglas-fir, and *P. menziesii* var. *glauca* (Beissner) Franco, called Rocky Mountain or interior Douglas-fir (Little 1979; Lipscomb 1993). The two varieties may intergrade in interior British Columbia (Canada) but have geographically separate ranges to the south. The varieties differ in many morphological, physiological and ecological characteristics. The coastal variety has greenish needles and longer seed cones with straight, appressed bracts, whereas the interior variety has bluish-green needles and shorter cones with reflexed bracts. Although the differences are not always obvious, strong differentiation of these varieties has been confirmed with isozymes, and nuclear, mitochondrial, and chloroplast DNA studies (Li and Adams 1989; Aagaard *et al*. 1995, 1998a, 1998b; Klumpp 1999; Nelson *et al*. 2003). Some of these studies have also indicated strong differentiation between the northern and southern subgroups of *P. menziesii* var. *glauca*. Literature particularly from Europe (*e.g.* Göhre 1958;

Klumpp 1999) sometimes recognizes another interior variety for the northern half of the continental range, *P. menziesii* var. *caesia* (Schwerin) Franco. Recently an additional variety, *P. menziesii* var. *oaxacana* Debreczy & Rácz, has been proposed for a few stands of narrow columnar trees with grayish foliage and small cones at the southernmost extent of the species' range (Debreczy and Rácz 1995; Acevedo-Rodríguez 1998).

2. Natural distribution

Douglas-fir is a common western North American species (central to southern area in both the Pacific and the Cordilleran regions), with a very broad latitudinal range extending from 55º N (Klinka *et al*. 2000; Hermann and Lavender 1990) to 16º N (Debreczy and Rácz 1995; Reyes-Hernández *et al*. 2005) (Figure 1). The coastal variety occurs from central British Columbia southward primarily along the Pacific Coast for about 2200 km to 34º44' N, reaching mid-California in the coastal Santa Cruz Mountains yet also occurring inland in the northern Sierra Nevada (Lipscomb 1993). The range of the continental interior variety extends along the Rocky Mountains into the mountains of southern Mexico over a distance of more than 4500 km. In northeastern Oregon, and southern Idaho southward through the mountains of eastern Nevada, Utah, Colorado, Arizona, New Mexico, western Texas and into Mexico, the distribution is discontinuous and fragmented. Disjunct populations are present in Alberta and the eastern-central parts of Montana and Wyoming (Hermann and Lavender 1990; Lipscomb 1993), and as far to the south as 16º22' N in central Oaxaca (Debreczy and Rácz 1995).

Figure 1. The main native range of Douglas-fir



Source: Hermann and Lavender 1990

3. Reproductive biology

3.1. Reproductive development

Douglas-fir is monoecious. Trees commonly reach reproductive maturity at 12 to 15 years of age. Primordia of undifferentiated buds are already present when vegetative buds flush in the spring of the year preceding the cone crop (Hermann and Lavender 1990). By mid-June, vegetative bud primordia, pollen cone primordia (usually clustered near the base of the extending shoot), and seed cone primordia (borne singly near the tip of the shoot) (Allen and Owens 1972) can be separated based on histochemical differences.

The number of lateral buds initiated that differentiate into reproductive buds, rather than aborting or developing into vegetative buds, determines the potential size of the cone crop. Poor seed cone crops in part reflect a high abortion rate of buds during the preceding summer. Large numbers of pollen cone buds or seed cone buds in the fall merely indicate the potential for a heavy cone crop the following year, as buds, cones and seeds can subsequently be damaged by frost and cones and seeds can be damaged or destroyed by seed predation before they mature (Dobbs *et al*. 1974).

Male strobili (pollen cones), generally abundant on year-old shoots especially in the lower crown, are about 2 cm long and yellow to deep red. Their overlapping microsporophylls each have two abaxial microsporangia (pollen sacs), which contain pollen mother cells that undergo meiosis and produce a tetrad of microspores. Each microspore develops into a mature, five-celled pollen grain containing two prothalial cells, a sterile (traditionally "stalk") cell, a generative (or "body") cell and a tube cell (Allen and Owens 1972; Fernando *et al*. 2005). The mature pollen grains are spherical when hydrated but dry and bowl-shaped when shed, have a seemingly smooth exine, and are rather large (90-110 μm diameter), so they do not disperse as far as the pollen of some conifers (Owens 1973; Tsukada 1982; Jackson and Smith 1994; Fernando *et al*. 2005). Under typical weather conditions most pollen is dispersed within ten tree heights, although small amounts can disperse over much greater distances when winds are strong. Pollen dispersal occurs for 20 to 30 days in a stand (Silen 1963).

Female strobili (seed cones), occur on year-old lateral shoots usually in the upper half of the crown. They are about 3 cm long, deep green to deep red, and have distinctive narrow trident bracts projecting from between the cone scales. The young cones are erect, and receptive to pollination when emerged (especially halfway or more) from the bud scales, *i.e.* at bud burst, and on average for 6-8 days (Webber and Painter 1996; Stein and Owston 2002). Anthesis and pollination of the coastal variety occur during March and April in warmer areas and as late as May or early June in colder areas. Pollen lands on the bracts and moves downward to the ovuliferous scales and inward to the apices of the inverted ovules (Takaso and Owens 1995). The cones then become larger and pendant, with the cones scales appressed.

Pollen collects on hairs of the integument's bilobed stigmatic tip, which then collapses inward to bring pollen into the micropyle (Allen and Owens 1972; Webber and Painter 1996). Fertilisation occurs about 10 weeks after pollination. *Pseudotsuga* and *Larix* have delayed ovular secretion, with a post-pollination pre-fertilisation drop filling the micropylar canal 5-7 weeks after pollination (von Aderkas and Leary 1999; Gelbart and von Aderkas 2002; Poulis *et al*. 2005). This drop is thought to assist the pollen grain in reaching the ovule and preparing for germination. The generative (body) cell of the pollen grain divides to produce two male gametes prior to fertilisation. After approximately 2 weeks, pollen grains germinate and pollen tubes grow into archegonia, releasing the two male gametes, one of which will fertilise the egg cell. Ovules contain four to six archegonia, thus multiple fertilisations and polyembryony occur, although typically only one embryo survives to maturity (Allen and Owens 1972). The embryo is in a cavity surrounded by the firm, cream-coloured, haploid megagametophyte which forms multinucleate storage cells, thus serving as a food reserve for the germinating embryo (Owens 1973; von Aderkas *et al*. 2005a). Each ovuliferous scale can produce two seeds at its base. Each seed (with the seed coat 5-7 mm long) has a large wing (twice to thrice the body length) consisting of two cell

layers derived from the ovuliferous scale. Mature seeds are dark brown on one side and mottled light brown on the other.

At low and middle elevations, cones mature and seeds ripen from mid-August to mid-September. The bracts turn brown when seeds are mature. Cone scales reflex and seedfall occurs under dry conditions soon after cone maturity, with two-thirds of the total crop typically on the ground by the end of October. Remaining seeds fall during the winter and spring. Reproductive phenology is similar for the interior variety (Baumgartner and Lotan 1991). The degree of dormancy of mature seed and thus the amount of chilling required to break it varies geographically. Chilling requirements are met over the winter and dormancy broken naturally. Dormancy is broken artificially through cold stratification; seeds are soaked in water for 24 hours, then chilled at 2 to 5°C, usually for 21 days. Coastal Douglas-fir generally requires cold stratification, whereas northern Rocky Mountain Douglas-fir may benefit from stratification and southern provenances may not. After dormancy is broken, seed will germinate at temperatures ranging from 10 to 30°C. There is no light requirement for germination. The viability of seed can be maintained for at least several decades when stored under optimal conditions, *i.e.* at 18ºC with a moisture content of 5 to 9% (Stein and Owston 2002).

3.2. Mating system and gene flow

Douglas-fir has a predominantly outcrossing mating system, with selfing rates generally well below 10% in natural populations, selectively harvested stands, and seed orchards (Neale and Adams 1985; Prat and Arnal 1994; Prat 1995; Prat and Caquelard 1995; Burczyk and Prat 1997; Slavov *et al*. 2005). Some trees may have considerably higher selfing rates than average (Stoehr *et al*. 1998).

Seed orchard studies of mating system and pollen contamination from outside stands indicate that two-thirds to half of the pollen received by a mother tree originates from nearby pollen parents, and a third to half comes from more remote sources, either within or outside of the orchard (Burczyk and Prat 1997; Slavov *et al*. 2005). Contamination of orchard seedlots with non-orchard pollen can be as high as 60% within the coastal Douglas-fir range (Adams *et al*. 1997). This contamination can result in decreased genetic gain or increased maladaptation, depending on the location and genetic makeup of the seed orchard (Stoehr *et al*. 1994).

Based on genetic markers, long-distance gene flow appears to be very low between the coastal and interior varieties, and between the northern and southern subgroups of the interior variety (Li and Adams 1989; Mitton 1992; Aagaard *et al*. 1995, 1998a, 1998b; Klumpp 1999; Nelson *et al*. 2003). However, the lack of strong differentiation of populations within the coastal variety (St. Clair *et al*. 2005), and within the northern subgroup of the interior variety, is indicative of higher levels of gene flow within each of these two relatively non-fragmented regions (Li and Adams 1989). Populations within the southern subgroup are much more isolated (Figure 1), although for example during the last glacial period there was considerably less fragmentation in the Colorado Plateau area and Rocky Mountains (Jackson *et al*. 2005).

3.3. Seed production

An old-growth Douglas-fir population may produce 20 to 30 times more cones per hectare than a 50- to 100-year-old second-growth stand. Seed crops occur at irregular intervals — on average, one heavy (mast) crop and one medium crop every 7 years (Owston and Stein 1974; Stein and Owston 2002). Even during heavy seed years, only about 25% of the trees produce an appreciable number of cones (Isaac 1943). Data on seed fall density in an area will vary widely, but most years less than 2.2 kg fall per ha, of which no more than 40% is viable. Years with small seed crops generally have a lower percentage of sound seeds than mast years, perhaps because the low density of seed and pollen-producing trees results in a higher level of self-pollination (Garman 1955).

Seed quality also varies during the annual seedfall period. It is higher in the fall of the year but declines rapidly during winter and spring. This pattern probably results from cone scales in the centre of the cone — where the highest quality seed are borne — opening early, and scales at the tip and base, which bear generally poor quality seeds, opening late (Hermann and Lavender 1990).

Both cones and seeds vary greatly in size with latitude and similarly in both varieties, generally with larger seeds to the south: for example, from coastal Douglas-fir trees, 112,000 cleaned seeds per kg in British Columbia, 70,000 per kg in California; from interior Douglas-fir trees, 110,000 seeds per kg in British Columbia, 71,000 per kg in Arizona (Stein and Owston 2002). Seeds of the coastal variety tend to be larger farther inland than near the coast, and seeds of the interior variety larger than in the coastal variety (*e.g.* 115,000 seeds per kg in western Washington, 88,000 per kg in Montana). Also, seeds sometimes are larger at higher elevations (Stein and Owston 2002). Individual cones can contain up to 52(-63) seeds, but an average of 15 to 20 seeds per cone is more typical in natural populations (Silen 1978; Vargas-Hernández *et al*. 2004). Seed size is determined before fertilisation, so there is no correlation between seed weight and paternity, although seedlings germinating from heavier seeds may be slightly larger during the first few months of growth (Hermann and Lavender 1990).

Predation of seed by insects, mammals and birds is a major factor limiting natural regeneration. Competing plant species and unfavourable abiotic environments also reduce success of regeneration. Although fully stocked stands have been reported 1 to 2 km away from a seed source, the vast majority of seeds fall within 100 m of seed parent trees (Allen 1942; Dobbs *et al*. 1974; Barnhart *et al*. 1996; Thompson and Schorn 1998; Brocano *et al*. 2005; Kennedy and Diaz 2005).

Most seed for artificial regeneration of coastal Douglas-fir now originates from seed orchards. Seed orchard managers promote the differentiation of reproductive buds through applications of synthetic giberellins (particularly giberellic acids GA_4 and GA_7), nitrate fertilisation, and partial girdling of stems in early spring (Silen 1978). Selected parents are grafted into seed orchards; graft incompatibility was a major problem until genetically high compatibility lines of rootstock were developed (Copes 1999). Cooling of orchards to delay heat sum accumulation and reproductive bud break is a common method for reducing pollen contamination in some orchards.

3.4. Natural regeneration

Douglas-fir seed germinate epigeally from mid-March to early April in the warmer regions of the range, and as late as mid-May in the cooler areas. Seedling growth during the first year is indeterminate, relatively slow, and moisture limited. Low moisture availability or a photoperiodic cue can trigger formation of buds and initiation of bud dormancy by mid-summer. Buds remain dormant until a genetically-determined chilling requirement is met sometime in winter, and growth resumes in April or May of the following year (Lavender 1984).

Seedlings of coastal Douglas-fir, particularly in wetter and cooler climates, survive best in high light when the seed germinates on moist mineral soil or thin burnt-over forest floor. In contrast, seedlings of interior Douglas-fir, particularly in drier and warmer climates, establish and survive in low light only on a relatively thick forest floor substrate (Isaac 1943; Schmidt 1957, 1969; Carter and Klinka 1992a). The establishment of seedlings is enhanced by ectomycorrhizal fungi (Horton *et al*. 1999; Cline *et al*. 2005). First-year seedlings on fresh sites may develop shoots 6 to 9 cm long. Growth in subsequent years is largely determinate, although some free growth and lammas growth occur in early years. Primary growth gradually accelerates so that when saplings are 8 to 10 years old, growth of the terminal leader may consistently exceed 1 m per yr on highly productive sites (Hermann and Lavender 1990). Lammas shoots of seedlings and saplings sometimes result in form defects such as forking and ramicorn branching, but these deviations are confined to wet climates with heavy late-summer rainfall (Carter and Klinka 1986; Carter *et al*. 1986; Hermann and Lavender 1990).

First-year germinants have higher survival and growth under light shade, especially on southerly exposures, but older seedlings require full sunlight, particularly on fresh and moist sites, where Douglas-fir has low shade tolerance (Carter and Klinka 1992a). Competing vegetation decreases light levels and limits Douglas-fir regeneration. On water-deficient sites, graminoids and shrubs also compete strongly with Douglas-fir seedlings for available moisture. Leaves and other organic debris from *Pteridium aquilinum* (northern bracken fern) and *Chamerion angustifolium* (narrow-leaf fireweed) can smother small seedlings. Regeneration may be more reliable after fire, which can destroy the seed bank of potential competing species, while forest harvesting can leave areas suitable for the establishment and growth of herbaceous and woody competitors (Burns 1983; Hermann and Lavender 1990).

Historically, large burned areas (including post-harvest burns) within the range of coastal Douglas-fir have naturally regenerated to nearly pure stands of Douglas-fir. On fresh sites, where Douglas-fir is considered an early seral and shade-intolerant species, this process occurred over a relatively short period, whereas on water-deficient and moist sites, regeneration has required more than 50 years. Regeneration on water-deficient sites in warmer and drier climates, where Douglas-fir is moderately shade-tolerant and considered a climax species, occurs under the forest canopy; in fact, temporary protection from the open-area climate is necessary for successful establishment (Daubenmire 1943; Krajina 1965; Ryker 1975; Hermann and Lavender 1990; Klinka *et al*. 1990).

From 1950 to 1970, large areas of cutover and burnt forest land in the Pacific Northwest were aerially seeded for reforestation. With the increase in forest nursery capacity and the ability to better control the success and density of regeneration through planting, direct seeding became rare (Schubert and Adams 1971; Cleary *et al*. 1978). One major problem with direct seeding is high seed predation by rodents and birds, although this problem can be reduced by seeding Douglas-fir seed mixed with a larger amount of desirable food such as sunflower seeds or oats (Sullivan and Sullivan 1984). Taking into account the range of sites on which Douglas-fir may grow and its variation in shade tolerance, it can be maintained using a wide variety of silvicultural systems — including clearcutting, seed-tree, shelterwood and selection systems. Although success of natural regeneration may be high, the advantage of planting is the opportunity for much greater initial stocking control and genetic improvement, particularly on productive sites. An adequate seed source, appropriate seedbed, and suitable microsite are all necessary for successful natural regeneration.

3.5. Vegetative reproduction

Douglas-fir does not reproduce vegetatively in nature. Reliable rooting of cuttings is limited to material collected from trees less than 10 years old, or from trees that have been subjected to repeated hedging which produces material with a juvenile habit. A second major impediment to the use of cuttings is that much of this material can have a lengthy period of plagiotropic growth before an erect habit is assumed (Hermann and Lavender 1990; Ritchie *et al*. 1994, 1997; Fennessy *et al*. 2000). Plagiotropic rooted cuttings become orthotropic more readily when grown outdoors than when maintained in a greenhouse; the reasons for this are not clear (Ritchie *et al*. 1997). Both rooted cuttings and plants produced through tissue culture of cotyledons appear more physiologically mature than seedlings of the same size (Ritchie *et al*. 1994). The rooting of stem cuttings is promoted through application of auxins, either IBA (24.6 mM) or NAA (2.5-7.4 mM) (Copes and Mandel 2000).

Methods for cloning Douglas-fir genotypes through somatic embryogenesis have been developed and are entering operational phases (Gadgil *et al*. 1998; Taber *et al*. 1998; Zhang *et al*. 1999; Benowicz *et al*. 2002). These methods allow for the development of clonal forestry programs, providing the technology to capture non-additive genetic variation, the opportunity to deploy clonal blocks for specific end uses, and a regeneration system for propagating genetically transformed material.

4. Genetics

4.1. Cytology

The 2C nuclear DNA content of *Pseudotsuga menziesii* is 38.10 picograms (O'Brien *et al*. 1996). The cell nuclear volumes are greater in northern provenances than southern provenances, and in the coastal variety than the interior variety (El-Lakeny and Sziklai 1971). Unlike all other species of the Pinaceae, the haploid number of chromosomes in Douglas-fir is 13. Whereas 5 metacentric and 6 submetacentric chromosomes appear karyotypically similar to those of other studied *Pseudotsuga* species, Douglas-fir has 2 telocentric chromosomes instead of a sixth large metacentric chromosome as in the other *Pseudotsuga* species. Moreover, the chromomycin A_3-banding pattern of Douglas-fir chromosomes is different from the quite similar banding patterns of the two Asian species (Hizume and Kondo 1992). Experimentally, polyploid Douglas-fir seedlings have been produced (using colchicine) but they were slow-growing and short-lived (Silen 1978).

In Douglas-fir, as in other species of the Pinaceae, inheritance of chloroplasts is predominantly paternal (Neale *et al*. 1986). In contrast, mitochondrial inheritance is maternal (Marshall and Neale 1992; Aagaard *et al*. 1998a).

4.2. Genetic variation

4.2.1. Population-level variability

Douglas-fir has substantial among-population variation both for quantitative traits and genetic markers. Population differentiation for quantitative traits related to adaptation to climate can be considerably stronger than that observed for selectively neutral genetic markers. For example, seedling studies including samples from a wide range of source environments have found that 13 to 20% of the total genetic variation for timing of bud set, 11 to 13% of the variation in timing of bud flush, and 47% of the variation for cold hardiness are attributable to differences among populations within varieties, which is higher than the average population differentiation for genetic markers (F_{ST}) (Table 1) (Howe *et al*. 2003). Short-term seedling genecological nursery studies have found significant differentiation of populations, particularly for bud phenology traits, over distances of only a few km or 100 m of elevation (Rehfeldt 1974, 1979a, 1979b; Campbell 1979, 1986). This variation is associated primarily with temperature of source environments, and generally reflects a tradeoff between growth rate and cold hardiness, which are negatively correlated among populations. The importance of this variation at local scales to the health and productivity of operational plantations has been a subject of debate in practice and the literature. Long-term field provenance trials within the native range and elsewhere have generally failed to show the fine-grained geographic patterns of variation observed in detailed seedling genecological trials (White and Ching 1985), fuelling a debate over the need to manage Douglas-fir genetic resources on a local scale (Stonecypher *et al*. 1996; Johnson 1997).

Field provenance trials have shown weaker geographic patterns than seedling studies. In a synthesis of the results from fifteen (mostly European) countries that established provenance trials from 1967 IUFRO seed collections, Breidenstein and collaborators (1990) identified four groups of provenance sites based on principal components analysis of climatic data: (1) sites in northeastern Europe with continental climates; (2) sites in northwestern British Columbia and Norway, along with a few locations in France and Spain, with cold maritime climates; (3) sites in southwestern British Columbia and northwestern Europe with moderate maritime climates; and (4) sites in southern Europe with warm maritime climates. Mortality was higher on sites in groups 1 and 2, and lower on sites in groups 3 and 4. There was substantial provenance variation in mortality among site groups, with trees from southern coastal Oregon suffering the highest mortality rates on the coldest (group 1) sites, and provenances from the interior of British Columbia having the highest mortality on the mildest (group 4) sites (Breidenstein *et al*. 1990).

However, surviving trees from low-elevation coastal and Cascade provenances in Washington State (USA) were surprisingly consistent in having the most rapid growth over most sites, showing broad adaptability, although mortality was high on some colder sites (Kleinschmit and Bastien 1992). Growth generally decreased with increasing source elevation of provenances. Some northern Oregon provenances from west of the Cascades as well as a few southwestern British Columbia sources also had high productivity across much of Europe. Only in continental climates, *e.g.* in Sweden, Finland and the Czech Republic, did *P. menziesii* var. *glauca* provenances outperform *P. menziesii* var. *menziesii*. However, marked changes in provenance performance between the ages of 17 and 60 in one of the oldest provenance trials, likely due to cumulative effects of small injuries as well as extreme climatic effects, caution against over-reliance on early field results (Silen 1978).

Table 1. Genetic diversity and population differentiation estimates for Douglas-fir

Marker type, and Population sampled	Expected heterozygosity (H_e) within populations	Population differentiation within races (G_{ST})	Population differentiation between races (G_{ST})	Reference
Allozymes				
Rangewide n=104 var. *menziesii* n=43 var. *glauca*: northern subgroup n=36 southern subgroup n=24	 0.164 0.150 0.077	 0.07 0.04 0.12	0.23	Li and Adams 1989
RAPD nuclear markers				
Rangewide n=6 var. *menziesii* n=2 var. *glauca*: northern subgroup n=2 southern subgroup n=2	 0.26 0.25 0.15	 0.05 0.07 0.25	0.25	Aagaard *et al*. 1998a
RAPD mitochondrial markers				
Rangewide n=6 var. *menziesii* n=2 var. *glauca*: northern subgroup n=2 southern subgroup n=2	 0.01 0.04 0.03	 0.04 0.75 0.84	0.72	Aagaard *et al*. 1998b

Although marker-based studies find less among-population variation than provenance and genecological studies, they confirm that Douglas-fir populations are relatively highly differentiated compared to various other tree species. A comprehensive rangewide survey of allozyme variation found that coastal and interior varieties were clearly differentiated, with an average Nei's genetic distance of 0.083, as were the interior variety's populations in northern and southern regions, separated by an average genetic distance of 0.034 (Li and Adams 1989). G_{ST} averaged 0.23 between varieties, 0.07 among populations within the coastal variety, 0.04 for the northern region of the interior variety, and 0.12 for the southern region of the interior variety (Table 1). These corresponded to average Nei's genetic distances among populations of respectively 0.015, 0.008, and 0.012. One of two Mexican populations was clearly distinct from all the other populations sampled. The strong differentiation of the coastal and interior varieties has been confirmed with nuclear RAPD (randomly amplified polymorphic DNA) markers. Mitochondrial RAPD markers exhibited even higher levels of racial and population differentiation (Aagaard *et al*. 1995, 1998a, 1998b), and restriction digestion of a region of chloroplast DNA also has shown strong racial differentiation (Nelson *et al*. 2003). Chloroplast restriction

fragment length polymorphisms (cpRFLPs) revealed less population differentiation (Ponoy *et al*. 1994). Chloroplast simple sequence repeats (cpSSRs) exhibited no differentiation among coastal British Columbia populations, presumably due to the recent migration into the region after glacial retreat and the long-distance gene flow *via* pollen within the region (Viard *et al*. 2001; Nelson *et al*. 2003).

4.2.2. Variation among individuals within populations

Levels of within-population variation are also high. The average expected heterozygosity (H_e) based on allozymes is high for coastal Douglas-fir (0.16) and the northern subgroup of interior Douglas-fir (0.15), but is half that for the more isolated southern interior subgroup (Li and Adams 1989) (Table 1). In contrast, for chloroplast DNA markers, levels of within-population variation are higher for interior populations than coastal populations (Ponoy *et al*. 1994). Mitochondrial RAPD markers revealed relatively low levels of within-population variation compared to nuclear RAPD markers, for which within-population variation was similar to allozymes (Aagaard *et al*. 1998b). The proportion of total genetic variation due to within-population variation is considerably higher for presumably selectively neutral molecular markers than for adaptive traits such as bud phenology (as predicted by theory) (Howe *et al*. 2003).

Table 2. Examples of individual heritability (h_i^2) estimates for quantitative traits in Douglas-fir

Trait	Variety	h_i^2 estimate, mean or range	Reference
Height 1 year	*menziesii*	0.60	Christophe and Birot 1979
Height 2 years	*menziesii*	0.46	Christophe and Birot 1979
Height 4 years	*menziesii*	0.26	Christophe and Birot 1979
Height 12 years	*menziesii*	0.15 (0.12-0.17)	Adams and Joyce 1990; Stonecypher *et al*. 1996
DBH 12 years	*menziesii*	0.08	Adams and Joyce 1990
Stem volume 12 years	*menziesii*	0.08	Adams and Joyce 1990
Lammas growth occurrence	*menziesii*	0.45	Aitken and Adams 1995a
	glauca	0.32	Rehfeldt 1983
Date of bud break	*menziesii*	0.87 (0.45-1.0)	Christophe and Birot 1979; Li and Adams 1993; El-Kassaby&Park 1993; Aitken&Adams 1995a
	glauca	0.52	Rehfeldt 1983
Date of bud set	*menziesii*	0.70 (0.15-0.81)	Li&Adams 1993; Aitken&Adams 1995a, 1995b
	glauca	0.25	Rehfeldt 1983
Fall cold hardiness	*menziesii*	0.29 (0.21-0.37)	Aitken and Adams 1996; Aitken *et al*. 1996; O'Neill *et al*. 2001
Winter cold hardiness	*menziesii*	0.11 (0.0-0.35)	Aitken and Adams 1995b
Spring cold hardiness	*menziesii*	0.62 (0.36-1.0)	Aitken and Adams 1997; O'Neill *et al*. 2001
Cambial growth initiation	*menziesii*	0.23	Li and Adams 1994
Cambial growth cessation	*menziesii*	0.11	Li and Adams 1994
Overall wood density	*menziesii*	0.59	Vargas-Hernandez and Adams 1991
Earlywood density	*menziesii*	0.47	Vargas-Hernandez and Adams 1991
Latewood density	*menziesii*	0.36	Vargas-Hernandez and Adams 1991
Latewood proportion	*menziesii*	0.24	Vargas-Hernandez and Adams 1991
Intra-ring density variation	*menziesii*	0.25	Vargas-Hernandez and Adams 1991
Graft incompatibility	*menziesii*	0.81	Copes 1973

The amount of genetic variation and the degree of genetic control of polygenic traits of economic or adaptive interest within populations of Douglas-fir have been studied extensively (Table 2). Generally, growth traits and fall cold hardiness are under relatively weak genetic control (individual heritability $h_i^2 < 0.3$) whereas bud phenology, spring cold hardiness, wood density and graft incompatibility are

under relatively strong genetic control ($h_i^2 \geq 0.5$). The polygenic control of bud phenology and cold hardiness traits has been verified through quantitative trait loci (QTL) mapping in a three-generation pedigree, with 11 QTL for fall cold hardiness, 15 for spring cold hardiness, and 33 for the timing of bud flush (Jermstad *et al*. 2001a, 2001b).

4.3. Inbreeeding depression and genetic load

Douglas-fir has a very high genetic load, with an estimated 10 lethal equivalents per genome (Sorensen 1969). Controlled outcrossing results in an average of 40 seeds per cone, whereas matings between half-sibs (inbreeding coefficient F=0.125) produce 31 seeds per cone, full-sib and parent-offspring matings (F=0.25) produce 23 seeds per cone, and self-pollination (F=0.50) averages just over one seed per cone (Sorensen and Cress 1994). Growth of seedlings and young trees is reduced by about 7% for every 0.1 increase in F (Sorensen 1997).

5. Hybridisation

Experimental hybrids between *Pseudotsuga menziesii* and *P. macrocarpa* have been reported (Ching 1959; Gause 1966; Orr-Ewing 1966a, 1966b). The present natural ranges of these species do not overlap, being about 35 km apart. Attempts to hybridise *P. menziesii* with generally accepted Asian species of the genus have failed, producing only empty seeds (Orr-Ewing 1966a, 1966b, 1971; Silen 1978). This failure is likely because of the different number of chromosomes in *P. menziesii* ($2n = 26$) from the diploid $2n = 24$ present in other species of the genus, but does not explain the interspecific crossing success with *P. macrocarpa* (Silen 1978). Attempts to hybridise *P. macrocarpa* with *P. japonica* and *P. sinensis* also have failed, producing only empty seeds (Orr-Ewing 1975).

Experimental crosses made in British Columbia of *Pseudotsuga flahaultii* (from western Chihuahua, Mexico) with *P. menziesii* var. *glauca* and *P. menziesii* var. *menziesii* (Orr-Ewing *et al*. 1972) were successful. The two generally accepted Douglas-fir taxa *P. menziesii* var. *menziesii* and *P. menziesii* var. *glauca* are completely interfertile experimentally. Intervarietal hybrids and F_2 crosses of such hybrids combine high growth rates with good frost resistance, and have been used in breeding programs in Germany (Braun 1992, 1999). Natural introgression of these two varieties may take place where their present ranges meet, for example in the interior of British Columbia (von Rudloff 1972; Li and Adams 1989), but in other areas of proximity the populations may remain distinct, possibly because of their local adaptation (*e.g.* St. Clair *et al*. 2005). When British Columbia was covered by the Cordilleran ice sheet 18,000 years ago, the varieties were isolated from each other farther to the south as coastal and Rocky Mountain populations; their convergence in interior British Columbia may have taken place no earlier than 7000 years ago (Tsukada 1982; Bartlein *et al*. 1998).

6. Ecology

This section focuses on the ecological information from Canada and the United States.

6.1. Autecology

Douglas-fir has an extensive geographical and elevational range, with the broadest ecological amplitude of all the western North American tree species. It grows in a wide variety of climates (arranged here in order of increasing frequency of presence): subalpine boreal, boreal, semiarid, temperate, and mesothermal (Hermann and Lavender 1990; Klinka *et al*. 2000). The Pacific region has a maritime climate with cool, relatively dry summers and wet, mild winters, with a long frost-free period and relatively narrow diurnal temperature fluctuations (6 to 8ºC). Precipitation falls mostly as rain, and is concentrated in the winter. The interior, Cordilleran region has a continental climate. In the northern part of the range of interior Douglas-fir, frost can occur in any month of the year. Precipitation in the northern

Rocky Mountains is relatively evenly distributed throughout the year, with the exception of a dry period during July and August. In the central Rocky Mountains, summers are hot and very dry in some areas, and winters are long and severe. The southern Rocky Mountains east of the Continental Divide generally have high rainfall during the growing season but low winter precipitation; west of the Continental Divide, the rainfall is bimodal, being more evenly divided between winter and summer. In the Sierra Madre Occidental of northern Mexico, the precipitation is primarily in the summer months, with occasional winter rainfall (but sometimes severe drought), average low temperature in January below freezing at higher elevations, and a spring dry period (Fulé and Covington 1999; Cleaveland *et al*. 2003; González-Elizondo *et al*. 2005).

Within these large regions the climate also varies considerably, which is readily notable with elevation. In general, temperature decreases and precipitation increases with increasing elevation throughout the Coastal ranges, Sierra Nevada, and Rocky Mountains. At middle and high elevations particularly north of Mexico, the winters are colder, the frost-free season is shorter, diurnal temperature fluctuations are larger (10 to 16ºC), and much of the precipitation falls as snow.

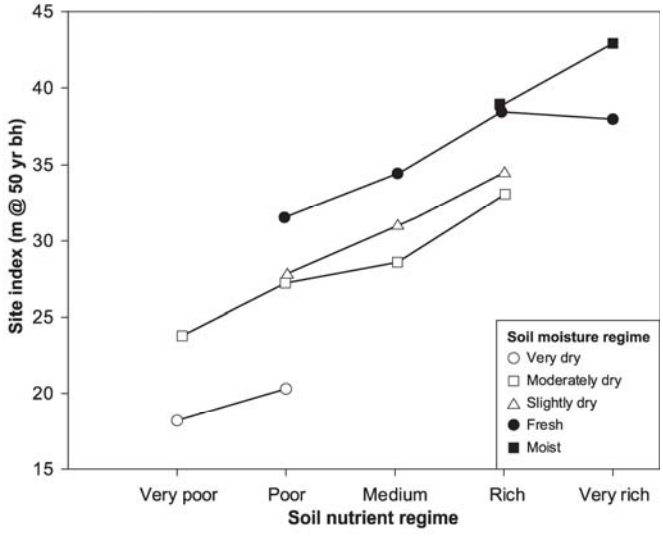
Douglas-fir grows across nearly the entire range of conditions of soil moisture (very dry to very moist) and soil nutrients (very poor to very rich), but the most productive growth occurs on fresh to moist, nitrogen-rich soils. Douglas-fir has a greater tolerance of water- and nutrient-deficient soils than many other native tree species (Krajina 1969; Klinka *et al*. 2000).

Site index is an expression of site productivity, based on the height of dominant or codominant trees at a standard base age (usually 50 years). Relationships between potential site index of the coastal variety and analytical and categorical measures of site quality have been quantified in the Coastal Western Hemlock zone of British Columbia (Klinka and Carter 1990). Site index increased with increasing soil water supply, peaked between fresh and moist sites, and then decreased with increasing water surplus. Site index also increased with increasing nitrogen availability, even on water-deficient sites (Figure 2). All the trends in site index – site quality relationships are supported by regression analysis indicating that each soil moisture and soil nutrient regime had a strong relationship with site index. The best quantitative soil measures related to site index were water deficit and mineralisable nitrogen in the first 30 cm of mineral soil, which together accounted for 63% of the Douglas-fir site index variability.

Both soil moisture and soil nitrogen are major determinants of Douglas-fir growth in the Coastal Western Hemlock zone (British Columbia) and likely in other environments (Carter and Klinka 1992b). Douglas-fir will respond to nitrogen fertilisation, with the magnitude of response decreasing with increasing soil water surplus and the available nitrogen (Carter *et al*. 1998) (Figure 3).

Coastal Douglas-fir reaches its best growth on well-aerated, deep soils with a pH from 5 to 6. In coastal northern California, Oregon and Washington, soils originated chiefly from marine sandstone and shales with scattered igneous intrusions. Surface soils are generally acidic, low in base saturation, and high in organic matter and total nitrogen. Soils supporting coastal Douglas-fir have textures ranging from gravelly sands to clays. Soil depth varies from very shallow on steep slopes and ridgetops, to deep where there are deposits of volcanic origin as well as residual and colluvial materials. Soil orders characteristic of the range of coastal Douglas-fir include Ultisols, Inceptisols, Spodosols and Entisols (Heilman *et al*. 1979).

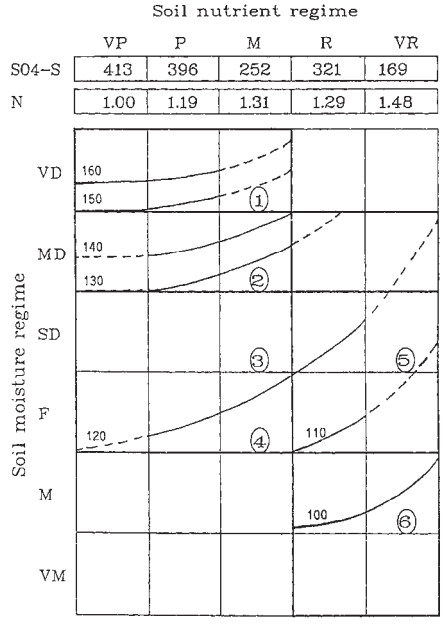
Figure 2. Douglas-fir site index relative to soil moisture and soil nutrient regimes in a dry, cool mesothermal climate of coastal British Columbia, Canada



Mineralizable-N (ppm)	7.3[d]	13.1[d]	25.2[c]	46.6[b]	176.5[a]
Foliar N (%)	1.14[a]	1.13[a]	1.19[a]	1.19[a]	1.24[a]
Mass of needles (mg)	470[b]	469[b]	479[b]	552[a]	610[a]

Relationships between soil nutrient regimes and several empirical variables (site n=149). Different superscripts within rows indicate significant differences among the soil nutrient regimes ($p<0.05$, Tukey's test) (from Carter and Klinka 1990)

Figure 3. Edatopic grid showing pre-treatment foliar nitrogen (N, %) and sulphate-sulphur (SO4-S, ppm), and isolines of relative basal-area response, indicating 3rd-year response of Douglas-fir to nitrogen fertilisation



Dashed isolines are extrapolations. Soil nutrient regimes (detailed in Figure 2) are: VD=very dry, MD=moderately dry, SD=slightly dry, F=fresh, M=moist, and VM=very moist. Encircled numbers are site units: 1=Cladonia spp., 2=Gaultheria shallon, 3=Rhytidiadelphus loreus, 4=Blechnum spicant, 5=Tiarella trifoliata, and 6=Athyrium filix-femina (from Carter and Klinka 1992b)

Soils in the range of interior Douglas-fir originated from an array of parent materials ranging from basaltic talus and deep loess with volcanic ash to thin residual soil over sedimentary or granitic rocks. The soils are mostly Alfisols, Inceptisols, Mollisols, Spodosols and Entisols. Limestone comprises a significant portion of the sedimentary rock, and gives rise to neutral or alkaline soils ranging in texture from gravelly silts to gravelly loams (Alexander 1974; Pfister *et al*. 1977).

The elevational distributions of both coastal and interior Douglas-fir decrease from south to north, reflecting the effect of latitude on temperature. Principal limiting factors are low temperature in the northern, high temperature in the far southern portions of the range, and low moisture especially in the southern portion (*e.g.* Adams and Kolb 2005; Case and Peterson 2005). Interior Douglas-fir generally grows at considerably higher elevations than coastal Douglas-fir at comparable latitudes. The highest elevation where interior Douglas-fir grows north of Mexico is 3260 m, on the crest of Mount Graham in southeastern Arizona (Hermann and Lavender 1990). Populations in Mexico generally occur between 2000-3600 m, on northern exposures (Acevedo-Rodríguez *et al*. 2006).

In summary, Douglas-fir tolerates water- and nutrient-deficient soils but not water-surplus and flooded soils (Krajina 1969; Klinka *et al*. 2000). In the Pacific Northwest and Intermountain Northwest, nitrogen is the only nutrient in forest soils that has been shown to limit the growth of Douglas-fir (Miller *et al*. 1986; Moore 1988).

Juvenile life-history characteristics are described above in Section III, Subsection D (Natural regeneration). Coastal Douglas-fir can reach reproductive maturity at 7 to 10 years of age, whereas Rocky Mountain Douglas-fir is slower to reproduce (Stein and Owston 2002). Sapling-age trees produce relatively few cones, investing most energy in rapid height growth under competition for light. Maximum fecundity occurs between 100 and 200 years, but younger trees produce fewer, larger cones and more viable seeds per cone (Stein and Owston 2002). The species produces relatively fewer cones in most years but large crops at intervals of 2 to 7 years (Owens 1973).

Coastal Douglas-firs commonly reach maximum heights of 76 m and diameters (dbh) of 150-180 cm. Among the living trees, one of the largest on record (1998) reached 85.6 m in height and 408 cm dbh, with a crown spread of 22 m, and wood volume of 308 m^3 (AFA 2000). The most massive recorded tree (1998) had a wood volume of 349 m^3 (height 73.8 m, dbh 423 cm, crown spread 23 m); and the tallest living tree (1998) reached 99.4 m (dbh 354 cm) (Van Pelt 2001). Historically, purportedly larger trees were reported, with heights of 115-127 m. Douglas-fir thus remains among the world's few tallest species. The lifespan of coastal Douglas-fir typically reaches some 500 years, with the oldest known tree attaining about 1350 years (McArdle *et al*. 1961; Hermann and Lavender 1990). Rocky Mountain Douglas-firs are smaller, averaging 30-37 m in height and 38-102 cm dbh, with one of the most massive recorded being 42.4 m in height and 255 cm dbh, and the tallest reaching 67.4 m with 179 cm dbh. The interior variety typically lives to around 400 years, although relatively frequent fires often kill trees at a younger age (Hermann and Lavender 1990); they can attain a known lifespan of some 1275 years. The Douglas-fir generations in an area can be overlapping or discrete, and long or short, depending on the agents of disturbance and whether the stands were even-aged or uneven in age.

6.2. Synecology

Depending on site and disturbance history, Douglas-fir can grow in even- or uneven-aged stands and in monospecific or mixed-species stands. It may be present in all seral stages of secondary succession, and can form old-growth stands on some sites.

As a result of its wide climatic amplitude, Douglas-fir is a minor or major component in many regional ecosystems (climatic or vegetation zones): for example, in British Columbia it occurs in 10 of the 12 forested zones. It is the major late seral species in the Interior Douglas-fir zone and Coastal Douglas-fir zone (Krajina 1965, 1969; Meidinger and Pojar 1991; Klinka *et al*. 2000). Given

its relatively wide edaphic amplitude, Douglas-fir can be a minor or major, temporary or self-perpetuating component of local ecosystems (plant associations, site types, habitat types, or forest cover types), but it is absent on wet sites, sites with a strongly fluctuating water table, and sites affected by ocean spray (Krajina 1969; Klinka *et al*. 2000).

The major associates of coastal Douglas-fir may include *Abies amabilis* (Pacific silver fir), *A. grandis* (grand fir), *A. concolor* (white fir), *Acer macrophyllum* (bigleaf maple), *Alnus rubra* (red alder), *Arbutus menziesii (*Pacific madrone), *Chamaecyparis lawsoniana* (Port Orford-cedar), *Libocedrus decurrens* (incense-cedar), *Picea sitchensis* (Sitka spruce), *Pinus monticola* (western white pine), *Quercus chrysolepis* (canyon live oak), *Q. garryana* (Garry oak), *Q. kelloggii* (California black oak), *Q wislizeni* (interior live oak), *Sequoia sempervirens* (coast redwood), *Thuja plicata* (western redcedar) and *Tsuga heterophylla* (western hemlock). Those species above that also occur in the Cordilleran region are also associated with interior Douglas-fir. Its other major associates are *Abies lasiocarpa* (subalpine fir), *Larix occidentalis* (western larch), *Picea glauca* (white spruce), *Pinus contorta* (lodgepole pine), *P. ponderosa* (ponderosa pine) and *Populus tremuloides* (trembling aspen) (Franklin and Dyrness 1973; Eyre 1980; Hermann and Lavender 1990).

Coastal Douglas-fir is a major component of four forest cover types (Eyre 1980): Pacific Douglas-fir (229), Douglas-fir–Western Hemlock (230), Port Orford Cedar (231) and Pacific Ponderosa Pine–Douglas-fir (244). It is a minor component of the following ten cover types: Red Alder (221), Sitka Spruce (223), Western Hemlock (224), Western Hemlock–Sitka Spruce (225), Coastal True Fir–Hemlock (226), Western Redcedar–Western Hemlock (227), Western Redcedar (228), Redwood (232), Oregon White Oak (233) and Douglas-fir–Tanoak–Pacific Madrone (234).

Interior Douglas-fir is a principal species in three forest cover types (Eyre 1980): Interior Douglas-fir (210), Western Larch (212) and Grand Fir (213). It is a minor component in five cover types: Engelmann Spruce–Subalpine Fir (206), White Fir (211), Western White Pine (215), Aspen (217) and Lodgepole Pine (218).

The cover and composition of understory vegetation within forest cover types vary depending on site (climate and soil), associated tree species, stand developmental stage, and stand density. Relative to other tree species, light interception by the canopy of Douglas-fir is intermediate, thus providing light conditions for the development of diverse understory vegetation.

Plantations of Douglas-fir in Europe, Argentina, Chile and New Zealand have been sources of natural reproduction for the naturalisation of Douglas-fir (MacLaren 1996; Knoerzer 1999; Ledgard and Langer 1999; Simberloff *et al*. 2003; Brocano *et al*. 2005). The rapid growth that has made Douglas-fir an exotic species of economic value with plantations in many areas may, in some cases, result in ecological problems as it can out-compete native species and potentially become invasive. There is some concern that conversion of native hardwood or grassland ecosystems to Douglas-fir dominated forests may result in changes in species composition, including insect communities, and soil acidity, fertility or nitrification (Alfredsson *et al*. 1998; Knoerzer 1999; Knoerzer and Reif 2001; Gossner and Simon 2002).

6.3. Stand dynamics

Periodic recurrence of major wildfire events has sometimes created large, rather pure stands of Douglas-fir, more so in the Pacific region than the Cordilleran region (*e.g.* Winter *et al*. 2002a, 2002b; Briles *et al*. 2005; Brunelle *et al*. 2005). The species' rapid growth and longevity, with thick corky bark of lower boles and main roots of older trees (*cf.* Kuiper and Coutts 1992; McConnon *et al*. 2004), and epicormic branching (Ishii and Ford 2001) are main adaptations that have enabled Douglas-fir to remain a dominant element in Pacific Northwest forests. Without major fire or other severe disturbance, Douglas-fir would gradually be replaced throughout much of this range by more shade-tolerant conifers. Although harvesting has reduced the area of the original old-growth forest, clearcutting with slash

burning followed by natural regeneration and/or planting has helped maintain Douglas-fir as the major component in second-growth stands. Where regeneration has been only partially successful or failed, *Pinus contorta*, broad-leaved trees or shade-tolerant conifers have become associates of Douglas-fir or replaced it altogether (Hermann and Lavender 1990). On the other hand, the historically recent lack of landscape-scale fire in some other areas of the western United States is causing encroachment of Douglas-fir into grasslands (Arno and Gruell 1986; Barnhart *et al*. 1996; Kennedy and Diaz 2005).

In Mexico, *Pseudotsuga* is a minor component in mixed-pine and *Abies* forests (*e.g.* Acevedo-Rodríguez 1998; Aguirre-Calderón *et al*. 2003; Domínguez-Álvarez *et al*. 2004). For example, it occurs in the Sierra Madre Oriental in *Abies vejarii* forests, and in Central Mexico with *A. religiosa*, and it is also found in association with *Pinus ayacahuite* and *P. hartwegii*.

The variation in shade tolerance of Douglas-fir from intolerant to moderately tolerant (Krajina 1965, 1969; Klinka *et al*. 1990; Carter and Klinka 1992a) is reflected in stand dynamics. In wetter and cooler climates (predominantly in the Pacific region, except on very dry sites in the rain shadow of the Olympic Mountains and southwestern Oregon and northern California), shade-intolerant Douglas-fir can be a minor or major but persistent seral species. Over several hundred years in the absence of stand-destroying events, it is replaced by shade-tolerant *Abies amabilis*, *Thuja plicata* and/or *Tsuga heterophylla* (Franklin and Dyrness 1973; Hermann and Lavender 1990). In drier and warmer climates (predominantly in the Cordilleran region, except the interior wet belt), moderately shade-tolerant Douglas-fir is a minor or major climax species: it is self-perpetuating under its own canopy. It replaces species such as *Pinus ponderosa*, *P. contorta* and *Larix occidentalis*. However, in the interior wet belt it functions as a minor or major seral species and is gradually replaced by shade-tolerant *Abies grandis*, *A. lasiocarpa*, *Picea engelmannii*, *Thuja plicata* and/or *Tsuga heterophylla* (Daubenmire 1943; Krajina 1969; Alexander 1988).

6.4. Damaging agents

Throughout life Douglas-fir is subject to damage from a variety of agents. It is host to hundreds of fungi, but relatively few cause serious damage. Over sixty species of insects attack Douglas-fir cones, but only a few result in significant damage to seed crops. Seed and cone insects include *Contarinia oregonensis* (Douglas-fir cone gall midge) and *C. washingtonensis* (Douglas-fir cone scale midge) (Diptera: Cecidomyiidae); *Leptoglossus occidentalis* (western conifer seed bug) (Hemiptera: Coreidae); *Megastigmus spermotrophus* (Douglas-fir seed chalcid) (Hymenoptera: Torymidae) (von Aderkas *et al*. 2005b); *Eupithecia spermaphaga* (fir cone looper) (Lepidoptera: Geometridae); *Dioryctria abietivorella*, *D. pseudotsugella* and *D. reniculelloides* (coneworms) (Lepidoptera: Pyralidae); *Barbara colfaxiana* (Douglas-fir cone moth) (Lepidoptera: Yponomeutidae); and *Frankliniella occidentalis* (western flower thrip) (Thysanoptera: Thripidae) (Hedlin *et al*. 1980). The damage by insects is frequently more pronounced during the years of light or moderate seed crops that follow mast crops (Furniss and Carolin 1977).

Various species of *Pythium* and *Rhizoctonia* (Peronosporales: Pythiaceae) and *Phytophthora*, *Fusarium* and *Botrytis* (Incertae sedis) fungi may cause significant seedling mortality in nurseries (Peterson and Smith 1975; Sutherland and van Eerden 1980). The root rots *Rhizina undulata* (Pezizales: Rhizinaceae), *Armillaria ostoyae* (Agaricales: Marasmiaceae) and *Phellinus weirii* (Hymenochaetales: Hymenochaetaceae) cause significant damage in plantations. The latter two fungi represent a serious threat to the management of young stands — trees either die or are weakened and blown over. The only effective control measures include removing infected stumps and introducing non-host species, and preclude continuous crop rotations of Douglas-fir. Many heart-rot fungi infect Douglas-fir; of these the most damaging and widespread is *Phellinus pini*, but *Phaeolus schweinitzii* (Polyporales: Polyporaceae) also causes serious problems. The main entry points for infection are knots and scars caused by fire, lightning and falling trees. Losses from heart rots far exceed those from any other type of decay

(Hermann and Lavender 1990). Other fungi found predominantly on dead wood of Douglas-fir include *Fomitopsis cajanderi* and *F. pinicola* (Polyporales: Fomitopsidaceae) (Hepting 1971).

Among needle diseases the most conspicuous is needle cast caused by *Rhabdocline pseudotsugae* (Heliotiales: Hemiphacidiaceae). It mainly affects younger trees, and typically only causes substantial damage after prolonged periods of rain while new needles are emerging. *Phaeocryptopus gaeumannii* (Pleosporales: Venturiaceae) needle blight is a serious problem in off-site plantations, especially in southern coastal Oregon. Serious stem deformities in the dry southern interior are caused by the dwarf mistletoe *Arceuthobium douglasii* (Santalales: Viscaceae), which occurs throughout most of the range of Douglas-fir (Hawksworth and Wiens 1996).

On interior Douglas-fir the most damaging instects are *Orgyia pseudotsugata* (Douglas-fir tussock moth) (Lepidoptera: Lymantriidae) and *Choristoneura fumiferana* (western spruce budworm) (Lepidoptera: Tortricidae). Both of these insects can attack trees of all ages at recurrent intervals (*e.g.* Campbell *et al*. 2005), and often result in severe defoliation. *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae) is a destructive bark beetle in old-growth stands of both coastal and interior Douglas-fir, but its impact is declining with the conversion to second-growth management and rotations of less than 100 years (Furniss and Carolin 1977).

Consumption of Douglas-fir seeds by small mammals and birds further impacts the quantity of seed available for regeneration. As most seedfall occurs at least 150 days before the germination period, the rodent *Peromyscus maniculatus* (deer mouse), can consume a great majority of the seed on the ground (Hermann and Lavender 1990). Browsing and clipping of seedlings and saplings by *Lepus americanus* (snowshoe hare), *L. townsendii* (jackrabbit), *Aplodontia rufa* (mountain beaver) and *Thomomys talpoides* (pocket gopher) often cause injury. *Odocoileus* spp. (deer) and *Cervus elaphus* (elk or wapiti) can also injure young trees (Black *et al*. 1979). In the Cordilleran region, domestic livestock can damage seedlings considerably through trampling and browsing.

High winds occasionally cause extensive blowdown of coastal Douglas-fir in the Pacific Northwest, particularly when following heavy rain. Scattered breakage of tree tops in dense, young stands can result from heavy snow and ice storms. Seedlings are vulnerable to both late spring and early fall frost events (Timmis *et al*. 1994). Interior Douglas-fir is less cold hardy than most sympatric conifers. Cold injury can be a concern with exotic plantations, for example in Europe and New Zealand (Hermann and Lavender 1999). In North America, crown fires can destroy stands of all ages; however, older Douglas-fir trees with thick bark are resistant to ground fires (Hermann and Lavender 1990).

7. Forestry practices

7.1. Deployment of reforestation materials

Douglas-fir is one of the most commonly regenerated trees in western North America, with the area planted now surpassing that regenerated naturally. Douglas-fir is grown in monospecific stands or mixed-species stands with shade-tolerant or shade-intolerant species. Depending on site and management objectives, clearcutting, seed-tree, shelterwood, and selection reproduction cuttings are effective silvicultural systems for its establishment and growth (Burns 1983). Propagation by seed is the primary method for regenerating the species. An overview of techniques for collection, processing, testing and storage of seed is in Stein and Owston (2002). Bare-root seedlings are predominantly used for artificial regeneration in the United States, and containerised seedlings in Canada. Most seed used for reforestation of coastal Douglas-fir comes from seed orchards. Interior Douglas-fir is largely regenerated by planting seedlings grown from wild-collected seed or is regenerated naturally, although young seed orchards will be providing more seed for the northern portion of the range (FGCBC 2001). Detailed information for pollen management is provided by Webber and Painter (1996).

Pseudotsuga menziesii has been a major component of western North American forests since the mid-Pleistocene (Hermann 1985). The known fossil record indicates that the species' native range did not extend beyond western North America, although *Pseudotsuga* fossils have been found in Alaska, Beringia and East Asia (Bartlein *et al*. 1998; Schorn and Thompson 1998). The arrival of David Douglas at Fort Vancouver (Washington State, USA) in April 1825 to collect seeds and plants for the Horticultural Society of London marked the beginning of the introduction of many North American species into Europe. He sent home many seeds and specimens, including Douglas-fir. Of all the North American species introduced into Europe, none has become more important (Hermann 1987; de Champs 1997). Plantations exist in many European countries ranging from Portugal to Russia, and Italy to Finland. The countries with the largest area of Douglas-fir plantations are France, where it is predicted to cover about 500,000 ha by 2010-2020, and Germany and Great Britain, where stands currently occupy about 80,000 ha and 50,000 ha, respectively. In the last 100 years Douglas-fir has been successfully introduced into many regions of the world's temperate forest zones, where it is grown in forests, arboreta and parks (Hermann 1987; Hermann and Lavender 1999).

7.2. Provenance transfer

The early introductions of Douglas-fir to Europe originated from coastal (*Pseudotsuga menziesii* var. *menziesii*) provenances, whereas some later seed imports from more interior regions (likely *P. menziesii* var. *glauca*) produced less successful plantations (Kleinschmit and Bastien 1992). Field provenance trials were first established in 1912 in Germany (Stimm and Dong 2001), and in 1913 in the Pacific Northwest (Munger and Morris 1936; Irgens-Möller 1968; Ching and Hermann 1977). Subsequently provenance trials were established in locations including Europe (Göhre 1958), New Zealand (Sweet 1964), Michigan (Steiner 1979), California (Griffin and Ching 1977), the Pacific Northwest (Ching and Bever 1960; Ching 1965; Rowe and Ching 1973; White and Ching 1985) and British Columbia (Haddock *et al*. 1967). The early literature on provenance variation was compiled by Hermann and Ching (1975). The early trials generally included relatively few provenances planted on relatively few sites, but still showed strong differentiation between the coastal and interior varieties in growth rate, frost hardiness, drought resistance and resistance to needle cast diseases (including *Rhabdocline pseudotsugae* and *Phaeocryptopus gaeumannii*) (Kleinschmit and Bastien 1992), which indicated the need for controlled seed transfer. Seed zones and seed transfer guidelines are designed to promote the utilisation of local, well-adapted and productive seedlots for reforestation by limiting movement of seed from the place of collection to the site for reforestation. Seed zones usually require that seedlots are collected and used within the same defined geographic area and elevational range, whereas seed transfer rules limit movement to some maximum latitudinal, longitudinal and elevational distance. For use as an exotic for reforestation or afforestation, seed transfer regulations define the area within the native range from which seed can be obtained.

In 1966 the International Union of Forest Research Organizations (IUFRO) initiated a systematic rangewide collection of 182 provenances of Douglas-fir, with seed distributed to 59 organisations in 36 countries to develop seed transfer rules for Europe (Kleinschmit and Bastien 1992). Provenance trials established from this collection varied in experimental design, number of provenances, and year planted, but have generated a wealth of information on optimal seed sources for use as an exotic (*e.g.* Breidenstein *et al*. 1990; Kleinschmit and Bastien 1992; Beran 1995; Kleinschmit *et al*. 1995; Kranenborg and de Vries 1995; Orlić and Ocvirek 1995; Vega *et al*. 1995). The results of these trials (summarised in Section IV. Genetics) have been used to develop seed transfer rules for European countries based on North American seed zones, to ensure that plantations are established from well-adapted, productive provenances (Kleinschmit and Bastien 1992). There is also considerable within-provenance variation for breeding programs to utilise, indicating that productive landraces can be developed from a range of provenances. Potential seed-collection areas for European forestry were defined following the IUFRO provenance trial (Fletcher *et al*. 1981; Fletcher and Bastien 1988, 1989 –

cited in Kleinschmit and Bastien 1992). The emphasis of Douglas-fir programs in western Europe has shifted from provenance selection to breeding programs using select genotypes from a range of appropriate sources.

Seed zones and seed-transfer guidelines for Douglas-fir within its native range are among the most conservative for western North American conifers. The guidelines are based in part on the relatively fine-scale geographic variation observed in seedling genecological trials (Rehfeldt 1974; Campbell 1979; Rehfeldt 1979a, 1979b; Campbell 1986). Field provenance trials have not shown the degree of local adaptation or the steepness of genetic clines that these seedling experiments have displayed. It is not clear if the differences in results between nursery and field trials are due to tree age, test environment, sampling issues, or experimental design problems (White and Ching 1985).

In coastal Oregon and Washington State, seed zones have recently been expanded slightly (Randall 1996; Randall and Berrang 2002). Douglas-fir still has more seed zones, and narrower elevational bands within zones (150 to 600 m), than other sympatric conifers in this region. In British Columbia, the seed planning zones are generally larger than in USA, and they are larger in the coastal region than the interior region. The maximum permitted distances for seed transfers of wild-stand seedlots of coastal Douglas-fir for reforestation within seed planning zones in British Columbia are up to 3º latitude to the north, up to 2º latitude to the south, within long (north to south) and narrow (east to west) seed planning zones, and 350 m up or down in elevation (BCMF 1995). Comparable maximum transfers for interior Douglas-fir within seed planning zones are 2º latitude north and 1º south, 3º longitude west and 2º east, and 200 m up in elevation and 100 m down.

7.3. Breeding programs

Breeding programs for coastal Douglas-fir are among the oldest and are the largest in the Pacific Northwest, with large numbers of progeny tests and seed orchards (Adams *et al*. 1990). Like seed zones, breeding zones for coastal Douglas-fir are generally long north-south and narrower east-west, reflecting coastal climatic gradients. The appropriate geographic size of breeding zones for coastal Douglas-fir has been controversial, due to the conflicting provenance and genecological test results described above. Some programs, such as the Pacific Northwest Tree Improvement Cooperative program, started with many small breeding zones based on the fine-scaled geographic variation observed in provenance trials and seedling genecological studies (Silen and Wheat 1979), and reinforced by quantification of substantial genotype-by-environment interaction in progeny trials (Campbell 1992). Other programs have delineated much larger breeding zones based on a lack of genotype-by-environment interaction in the field growth of highly ranked families and a lack of correspondence between genotype-by-environment interaction and variation in physical environments between test sites (Stonecypher *et al*. 1996; Johnson 1997).

Early breeding programs focused on obtaining genetic gain through intensive phenotypic selection in wild stands. This resulted in gains of a few percent for juvenile growth (Stonecypher *et al*. 1996). Subsequent progeny testing and selection resulted in gains of around 10% for growth rate in the first generation. Some breeding programs used open-pollinated progeny trials, whereas others had progeny testing of extensive partial diallel matings. In the latter, the amount of non-additive genetic variation for traits of interest was found to be about half that of additive genetic variation (Stonecypher *et al*. 1996), although this varies greatly among sets of genotypes (Yanchuk 1996). Thus, programs focus primarily on utilising additive variation. As cloning technology improves (*e.g.* somatic embryogenesis), interest may increase in capturing some non-additive variation through deployment of clones in some situations.

The objectives for these selective breeding programs are to increase volume growth while maintaining quality traits including stem form, wood density and branch diameter. In western Europe, increasing spring cold hardiness through delaying bud burst is also of interest (*e.g,*. Heois 1994). Most programs are currently in the second generation of breeding and testing. Selection for faster growth

can lead to a greater frequency of lammas growth on highly productive sites, and this can produce forking and ramicorn branching defects (Adams and Bastien 1994; Schermann *et al*. 1997), so lammas growth is selected against in some programs. Some breeding lines within multiple-population breeding programs focus on increasing wood density. Stem volume and wood density are moderately negatively genetically correlated, so simultaneous improvement of these traits is difficult. Breeding efforts for interior Douglas-fir in British Columbia, Idaho and Montana are more recent and smaller in scale than their coastal counterparts, but also focus on increasing wood volume, with wood density as a secondary consideration (FGCBC 2001). Cooperative European breeding efforts have arisen from the IUFRO provenance trial among France, Belgium, Spain and Germany, with a base population of 10 provenances and 50 open-pollinated progeny from each (Kleinschmit and Bastien 1992).

Estimates of the optimum age for genetic selection for increased growth rate have ranged from 4 years in highly cultivated, intensive farm field tests up to 7 to 18 years in some field trials (Magnussen and Yanchuk 1994; Woods *et al*. 1995; Johnson *et al*. 1998). Seedlings 1 or 2 years of age can be used to identify the poorest families and cull genotypes prior to establishing progeny trials, but the best families cannot be identified in early tests (Adams *et al*. 2001). Final selections have typically been made in field trials at 12 to 15 years. Spacing of trees or testing genotypes in mixtures *versus* pure blocks does not significantly affect estimation of genetic parameters or genotype rankings (St. Clair and Adams 1991; El-Kassaby and Park 1993).

7.4. Conservation of genetic resources

The inherently high genetic diversity of Douglas-fir, both within and among populations, is being conserved both *in situ* in natural parks, ecological reserves and other protected areas across most of the species' range, and *ex situ* in seed orchards, breeding archives and genetic field tests. Geographic information system-based spatial analyses of the adequacy of *in situ* protection of Douglas-fir in parks have recently been conducted in British Columbia, Washington and Oregon, and its ecological envelope has also been modelled in relation to protected areas (Coulston and Riitters 2005). In British Columbia, *in situ* protection was analyzed for each of twelve Seed Planning Units (SPUs) used for managing seed transfer and breeding programs (Hamann *et al*. 2004). Only protected areas over 250 ha with at least 5000 mature-equivalent individuals were considered adequate reserves. The number of protected areas meeting these criteria in each SPU ranged from two to sixty. Nearly all of the SPUs appeared to have sufficient conservation populations, with the Cariboo Transition SPU near the northern edge of the *Pseudotsuga menziesii* var. *glauca* range being the only area judged to need field verification of adequacy of protection.

In Washington and Oregon, the analyses were conducted based on both seed zones and ecoregions. The conservation threshold set was that at least 5000 reproductively mature individuals be protected in parks and ecological reserves in each spatial unit (Lipow *et al*. 2004). Of 204 seed zone-by-elevation bands in Washington and western Oregon, 198 were well protected. The primary *in situ* conservation gap was in the Puget Lowlands of western Washington State (Lipow *et al*. 2004; Coulston and Riitters 2005), an area of high forest productivity. Fortunately genotypes from this region are well represented in *ex situ* collections (Lipow *et al*. 2004). Provenances from the region are well represented and important in breeding populations in western Europe (Kleinshmidt and Bastien 1992). In eastern Oregon, 14 of 18 breeding zone-by-elevation bands were also considered well protected; conservation gaps were identified in the Fort Rock and Chiloquin breeding zones, but unprotected stands of Douglas-fir in these mid-southern areas were considered unlikely to be harvested. Additional protected areas were considered desirable for the species in northwestern California (Coulston and Riitters 2005), and greater protection for populations in Mexico (Vargas-Hernández *et al*. 2004).

In addition to *in situ* conservation reserves and extensive *ex situ* resources in seed banks, provenance and progeny trials, and breeding populations within the native range, there are considerable *ex situ*

collections of Douglas-fir in both North America and Europe. In France, Germany and Belgium, over 1000 ha of *ex situ* gene conservation plantations have been established from provenances of interest in USA (Kleinschmidt and Bastien 1992). The widespread use of relatively local seed and breeding zones to control the movement of seed for reforestation within the native range also maintains adaptation and geographic variation in reforested areas.

Many studies have evaluated genetic diversity of Douglas-fir resulting from forest management practices, and generally have found little change relative to wild populations. Alternative regeneration methods have been compared, including shelterwood systems, group selection and clearcutting followed by natural regeneration or planting (Adams *et al*. 1998). In general, harvesting and regeneration methods had little effect on genetic diversity, although harvesting from below (removing smaller trees) in the shelterwood method resulted in removal of some rare, possibly deleterious alleles. Similarly, the silvicultural system had little effect on the mating system (Neale and Adams 1985). First-generation and second-generation domesticated populations of coastal Douglas-fir in British Columbia and Washington have similar or higher levels of genetic diversity than wild populations, although second-generation populations differ slightly for some allele frequencies from wild populations (El-Kassaby and Ritland 1996a, 1996b). It has been suggested that slightly higher levels of genetic diversity would be maintained through nursery production of seedlings if single seeds were sown from bulked seedlots or if individual families were managed separately (El-Kassaby and Thomson 1996).

8. Summary

Douglas-fir is one of the most important and valuable timber species globally. Its wood is moderately heavy and hard, and exceptionally strong. It is a source of wood for both lumber and pulp, and used for structural purposes, in shipbuilding, and in the production of items such as laminated beams and interior and exterior finishing, boxes, railway ties, and when impregnated with a preservative, in piling and decking for marine structures. Douglas-fir is also grown for seasonal Christmas trees, and as an ornamental.

Across its native range in western North America, Douglas-fir is a long-lived and ecologically important species. It is a seral species in wet and cool climates, and a fire-adapted climax species in dry and warm climates. Because of its rapid growth rate, it produces a higher volume of wood sooner than many of its associates, and is valuable as an exotic plantation species in many temperate regions. It has moderate nutrient requirements and is easy to regenerate and grow. The ecology of Douglas-fir is diverse, in keeping with its large geographical distribution. It may grow in pure, single-storied, even-aged stands as well as in multi-aged and multi-storied stands. It is associated with many softwood and hardwood species in diverse ecosystems throughout a considerable range of climatic zones. Douglas-fir is also a major tree species in critical watersheds and in many scenic and recreational areas. It is a component of a very large area of wildlife habitat, and is widely associated with grazing and range allotments.

Douglas-fir has very high levels of genetic diversity, and this variation represents an important resource. Genetic clines are strong, related primarily to environmental gradients in temperature, but also to moisture. There is a lack of consensus on how narrowly populations are adapted, and thus at what geographic scale they should be managed. Genetic diversity in this species is fairly well protected in most regions, both *in situ* and *ex situ*, and potential conservation gaps in the northernmost and northwestern portions of the range have been assessed. Breeding programs, particularly for the coastal variety, are large and well into the second generation of domestication. Selective breeding is increasing growth rate while maintaining the stem form and wood quality that make this such a desirable timber species. New cloning technologies, primarily somatic embryogenesis, allow for consideration of clonal strategies for improving this species.

References

Aagaard, J.E., K.V. Krutovskii and S.H. Strauss. 1998a. RAPD markers of mitochondrial origin exhibit lower population diversity and higher differentiation than RAPDs of nuclear origin in Douglas-fir. *Molecular Ecology* 7: 801-812.

Aagaard, J.E., K.V. Krutovskii and S.H. Strauss. 1998b. RAPDs and allozymes exhibit similar levels of diversity and differentiation among populations and races of Douglas-fir. *Heredity* 81: 69-78.

Aagaard, J.E., S.S. Vollmer, F.C. Sorensen and S.H. Strauss. 1995. Mitochondrial DNA products among RAPD profiles are frequent and strongly differentiated between races of Douglas-fir. *Molecular Ecology* 4: 441-446.

Acevedo-Rodríguez, R. 1998. Estudio Sinecológico del Bosque de *Pseudotsuga menziesii* (Mirb.) Franco var. *oaxacana* Debreczy & Rácz, en la Zona de Santa Catarina Ixtepeji, Oaxaca, México. Tesis Profesional, División de Ciencias Forestales, Universidad Autónoma Chapingo, Chapingo, Mexico. 105 pp.

Acevedo-Rodríguez, R., J.J. Vargas-Hernández, J. López-Upton and J. Velázquez-Mendoza. 2006. Efecto de la procedencia geográfica y de la fertilización en la fenología del brote terminal en plántulas de *Pseudotsuga* sp. / Effect of geographic origin and nutrition on shoot phenology of Mexican Douglas-fir (*Pseudotsuga* sp.) seedlings. *Agrociencia (Montecillo)* 40: 125-137.

Adams, H.D., and T.E. Kolb. 2005. Tree growth response to drought and temperature in a mountain landscape in northern Arizona, USA. *Journal of Biogeography* 32: 1629-1640.

Adams, W.T., and J.-C. Bastien. 1994. Genetics of second flushing in a French plantation of coastal Douglas-fir. *Silvae Genetica* 43: 345-352.

Adams, W.T., and D.G. Joyce. 1990. Comparison of selection methods for improving volume growth in young coastal Douglas-fir stands. *Silvae Genetica* 39: 219-226.

Adams, W.T., S.N. Aitken, D.G. Joyce, G.T. Howe and J. Vargas-Hernández. 2001. Evaluating efficacy of early testing for stem growth in coastal Douglas-fir. *Silvae Genetica* 50: 167-175.

Adams, W.T., V.D. Hipkins, J. Burczyk and W.K. Randall. 1997. Pollen contamination trends in a maturing Douglas-fir seed orchard. *Canadian Journal of Forest Research* 27: 131-134.

Adams, W.T., G. Johnson, D.L. Copes, J. Daniels, R.E. Quam, J.C, Heaman and J. Weber. 1990. Is research keeping up with the needs of Douglas-fir tree improvement programs? *Western Journal of Applied Forestry* 5: 135-137.

Adams, W.T., J.H. Zuo, J.Y. Shimizu and J.C. Tappeiner. 1998. Impact of alternative regeneration methods on genetic diversity in coastal Douglas-fir. *Forest Science* 44: 390-396.

AFA (American Forestry Association). 2000. The National Register of Big Trees, 2000-01. *American Forests* 106(1): 22-64.

Aguirre-Calderón, Ó.A, J. Jiménez-Pérez, H. Kramer and A. Akça. 2003. Análisis estructural de ecosistemas forestales en el Cerro del Potosí, Nuevo León, México. *Ciencia UANL* 6: 219-225.

Aitken, S.N., and W.T. Adams. 1995a. Impacts of genetic selection for height growth on annual developmental cycle traits in coastal Douglas-fir. AD1: 1-8 *in* Evolution of Breeding Strategies for Conifers from the Pacific Northwest. Proceedings, Joint Meeting of IUFRO Working Parties S2.02.05 (Douglas-fir), S2.02.06 (*Pinus contorta*), S2.02.12 (Sitka spruce) and S2.02.14 (*Abies*), 1 -4 August, Limoges, France. INRA (Institut National de la Recherche Agronomique), Station d'Amélioration des Arbres Forestiers, Ardon, France.

Aitken S.N., and W.T. Adams. 1995b. Screening for cold hardiness in coastal Douglas-fir. Pp. 321-324 *in* B.M. Potts, N.M.G. Borralho, J.B. Reid, R.N. Cromer, W.N. Tibbits and C.A. Raymond, eds. Proceedings of the CRC/IUFRO Conference, 19-24 February 1995, Hobart, Australia.

Aitken, S.N., and W.T. Adams. 1996. Genetics of fall and winter cold hardiness of coastal Douglas-fir in Oregon. *Canadian Journal of Forest Research* 26: 1828-1837.

Aitken, S.N., and W.T. Adams. 1997. Spring cold hardiness under strong genetic control in Oregon populations of *Pseudotsuga menziesii* var. *menziesii*. *Canadian Journal of Forest Research* 27: 11773-11780.

Aitken, S.N., W.T. Adams, N. Schermann and L.H. Fuchigami. 1996. Family variation for fall cold hardiness in two Washington populations of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco). *Forest Ecology and Management* 80: 187-195.

Alexander, R.R. 1974. Silviculture of Subalpine Forests in the Central and Southern Rocky Mountains: The Status of Our Knowledge. USDA Forest Service Research Paper RM-121. Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado. 88 pp.

Alexander, R.R. 1988. Forest Vegetation on National Forests in the Rocky Mountain and Intermountain Regions: Habitat Types and Community Types. USDA Forest Service Research Paper RM-162. Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado. 47 pp.

Alfredsson, H., L.M. Codron, M. Clarholm and M.R. Davis. 1998. Changes in soil acidity and organic matter following the establishment of conifers on former grasslands in New Zealand. *Forest Ecology and Management* 112: 245-252.

Allen, G.S. 1942. Douglas-Fir (*Pseudotsuga taxifolia* (Lamb.) Britt.): A Summary of its Life History. British Columbia Forest Service, Research Notes 9. 27 pp.

Allen, G.S., and J.N. Owens. 1972. The Life History of Douglas-fir. Forestry Service, Environment Canada, Ottawa. 139 pp.

Arno, S.F., and G.E. Gruell. 1986. Douglas-fir encroachment into mountain grasslands in southwestern Montana. *Journal of Range Management* 39: 272-275.

Barnhart, S.J., J.R. McBride and P. Warner. 1996. Invasion of Northern Oak Woodlands by *Pseudotsuga menziesii* (Mirb.) Franco in the Sonoma Mountains of California. *Madroño* 43: 28-45.

Bartlein, P.J., K.H. Anderson, P.M. Anderson, M.E. Edwards, C.J. Mock, R.S. Thompson, R.S. Webb, T. Webb III and C. Whitlock. 1998. Paleoclimate simulations for North America over the past 21,000 years: Features of the simulated climate and comparisons with paleoenvironmental data. *Quaternary Science Reviews* 17: 549-585.

Baumgartner, D.M., and J.E. Lotan, compilers. 1991. Interior Douglas-fir: The Species and its Management. Symposium Proceedings, February 27-March 1, 1990, Spokane, Washington. Washington State University Cooperative Extension MISC0230, Pullman, Washington. 301 pp.

BCMF. 1995. Seed and Vegetative Material Guidebook. Forest Practices Code, British Columbia Ministry of Forests [BCMF], Victoria, B.C. 77 pp.

Benowicz, A., S.C. Grossnickle and Y.A. El-Kassaby. 2002. Field assessment of Douglas-fir somatic and zygotic seedlings with respect to gas exchange, water relations, and frost hardiness. *Canadian Journal of Forest Research* 32: 1822-1828.

Beran, F. 1995. Hitherto results of Douglas-fir provenance research in the Czech Republic. DF6: 1-17 *in* Evolution of Breeding Strategies for Conifers of the Pacific Northwest. Proceedings of the Joint Meeting of IUFRO Working Parties S2.02.05 (Douglas-fir), S2.02.06 (*Pinus contorta*), S2.02.12 (Sitka spruce) and S2.02.14 (*Abies*), 1 -4 August, Limoges, France. INRA, Station d'Amélioration des Arbres Forestiers, Ardon, France.

Black, H.C., E.J. Dimock II, J. Evans and J.A. Rochelle. 1979. Animal damage to coniferous plantations in Oregon and Washington. Research Bulletin 25, School of Forestry, Oregon State University, Corvallis. 45 pp.

Braun, H. 1992. Some results of Douglas-fir breeding at Graupa. *Silvae Genetica* 41: 188-195.

Braun, H. 1999. Douglas-fir breeding in Saxony. *Silvae Genetica* 48: 69-77.

Breidenstein, J., J.-C. Bastien and B. Roman-Amat. 1990. Douglas-fir range-wide variation results from the IUFRO Data Base. 2.13: 1-14 *in* Proceedings, IUFRO Meeting of Working Party S2.02.05 (Douglas-fir), August 1990, Olympia, Washington.

Briles, C.E., C. Whitlock and P.J. Bartlein. 2005. Postglacial vegetation, fire, and climate history of the Siskiyou Mountains, Oregon, USA. *Quaternary Research* 64: 44-56.

Brocano, M.J., M. Vilà and M. Boada. 2005. Evidence of *Pseudotsuga menziesii* naturalization in montane Mediterranean forests. *Forest Ecology and Management* 211: 257-263.

Brunelle, A., C. Whitlock, P. Bartlein and K. Kipfmueller. 2005. Holocene fire and vegetation along environmental gradients in the Northern Rocky Mountains. *Quaternary Science Reviews* 24: 2281-2300.

Burczyk, J.L., and D. Prat. 1997. Male reproductive success in *Pseudotsuga menziesii* (Mirb.) Franco: The effects of spatial structure and flowering characteristics. *Heredity* 79: 638-647.

Burns, R.M., tech. compiler. 1983. Silvicultural Systems for the Major Forest Types of the United States. USDA Agriculture Handbook 445, Washington, D.C.

Campbell, R., D.J. Smith and A. Arsenault. 2005. Dendroentomological and forest management implications in the Interior Douglas-fir zone of British Columbia, Canada. *Dendrochronologia* 22: 135-140.

Campbell, R.K. 1979. Genecology of Douglas-fir in a watershed in the Oregon Cascades. *Ecology* 60: 1036-1050.

Campbell, R.K. 1986. Mapped genetic variation of Douglas-fir to guide seed transfer in southwest Oregon. *Silvae Genetica* 35: 85-96.

Campbell, R.K. 1992. Genotype × Environment Interaction: A Case Study for Douglas-fir in Western Oregon. USDA Forest Service Research Paper PNW-RP-455. Pacific Northwest Research Station, Portland, Oregon. 21 pp.

Carter, R.E., and K. Klinka. 1986. Symptoms and Causes of Distorted Growth in Immature Forest Stands in Coastal British Columbia. Land Management Report 39, British Columbia Ministry of Forests, Victoria, B.C. 61 pp.

Carter, R.E., and K. Klinka. 1990. Relationships between growing-season soil water-deficit, mineralizable soil-nitrogen and site index of coastal Douglas-fir. *Forest Ecology and Management* 30: 301-311.

Carter, R.E., and K. Klinka. 1992a. Inter- and intraspecific variation in shade tolerance of Douglas-fir, western hemlock, and western redcedar. *Forest Ecology and Management* 55: 87-105.

Carter, R.E., and K. Klinka. 1992b. Use of ecological site classification in the prediction of forest productivity and response to fertilization. *Suid-Afrikaanse Bosboutydskrif* 160: 19-23.

Carter, R.E., A.M. Scagel and K. Klinka. 1986. Nutritional aspects of distorted growth in immature forest stands of coastal southwestern British Columbia. *Canadian Journal of Forest Research* 16: 36-41.

Carter, R.E, E.R.G. McWilliams and K. Klinka. 1998. Predicting response of Douglas-fir to fertilizer treatments. *Forest Ecology and Management* 107: 275-289.

Case, M.J., and D.L. Peterson. 2005. Fine-scale variability in growth-climate relationships of Douglas-fir, North Cascade Range, Washington. *Canadian Journal of Forest Research* 35: 2743-2755.

Ching, K.K. 1959. Hybridization between Douglas-fir and big-cone Douglas-fir. *Forest Science* 5: 246-254.

Ching, K.K. 1965. Early Growth of Douglas-fir in a Reciprocal Planting. Forest Research Laboratory Research Paper 3, Oregon State University, Corvallis. 21 pp.

Ching, K.K., and D. Bever. 1960. Provenance study of Douglas-fir in the Pacific Northwest region. I. Nursery performance. *Silvae Genetica* 9: 11-17.

Ching, K.K., and R.K. Hermann. 1977. Intraspecific variations in Douglas-fir. *Genetics Lectures* 5: 19-32.

Christophe, C., and Y. Birot. 1979. Genetic variation within and between populations of Douglas-fir. *Silvae Genetica* 28: 197-206.

Cleary, B.D., R.D. Greaves and R.K. Hermann. 1978. Regenerating Oregon's Forests. A Guide for the Regeneration Forester. Oregon State University, Extension Services, Corvallis. 286 pp.

Cleaveland, M.K., D.W. Stahle, M.D. Therrell, J. Villanueva-Díaz and B.T. Burns. 2003. Tree-ring reconstructed winter precipitation and tropical teleconnections in Durango, Mexico. *Climatic Change* 59: 369-388.

Cline, E.T., J.F. Ammirati and R.L. Edmonds. 2005. Does proximity to mature trees influence ectomycorrhizal fungus communities of Douglas-fir seedlings? *New Phytologist* 166: 993-1009.

Copes, D.L. 1973. Inheritance of graft compatibility in Douglas-fir. *Botanical Gazette* 134: 49-52.

Copes, D.L. 1999. Breeding graft-compatible Douglas-fir rootstocks (*Pseudotsuga menziesii* (Mirb.) Franco). *Silvae Genetica* 48: 188-193.

Copes, D.L., and N.L. Mandel. 2000. Effects of IBA and NAA treatments on rooting Douglas-fir stem cuttings. *New Forests* 20: 249-257.

Coulston, J.W., and K.H. Riitters. 2005. Preserving biodiversity under current and future climates: A case study. *Global Ecology and Biogeography* 14: 31-38.

Daubenmire, R. 1943. Soil temperature versus draught as a factor determining lower altitudinal limits of trees in the Rocky Mountains. *Botanical Gazette* 105: 1-13.

Debreczy, Z., and I. Rácz. 1995. New species and varieties of conifers from Mexico. *Phytologia* 78: 217-243.

de Champs, J., coord. 1997. Le Douglas. AFOCEL (Association Forêt-cellulose), Paris. 416 pp.

Dobbs, R.C., D.G.W. Edwards, J. Konishi and D. Wallinger. 1974. Guideline to Collecting Cones of British Columbia Conifers. Joint Report 3, British Columbia Forest Service & Canadian Forestry Service, Victoria, B.C. 98 pp.

Domínguez-Álvarez, F.A., J.J. Vargas-Hernández, J. López-Upton, P. Ramírez-Vallejo and E. Guízar-Nolazco. 2004. Aspectos ecológicos de *Pseudotsuga menziesii* en el ejido La Barranca, Pinal de Amoles, Querétaro. *Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Botánica* 75: 191-203.

Elias, T.S. 1980. The Complete Trees of North America: Field Guide and Natural History. Van Nostrand Reinhold, New York. 948 pp.

El-Kassaby, Y.A., and Y.S. Park. 1993. Genetic variation and correlation in growth, biomass, and phenology of Douglas-fir diallel progeny at different spacings. *Silvae Genetica* 42: 289-297.

El-Kassaby, Y.A., and K. Ritland. 1996a. Genetic variation in low elevation Douglas-fir of British Columbia and its relevance to gene conservation. *Biodiversity and Conservation* 5: 779-794.

El-Kassaby, Y.A., and K. Ritland. 1996b. Impact of selection and breeding on the genetic diversity in Douglas-fir. *Biodiversity and Conservation* 5: 795-813.

El-Kassaby, Y.A., and A.J. Thomson. 1996. Parental rank changes associated with seed biology and nursery practices in Douglas-fir. *Forest Science* 42: 228-235.

El-Lakeny, M.H., and O. Sziklai 1971. Intraspecific variation in nuclear characteristics of Douglas-fir. *Advancing Frontiers of Plant Science* 28: 363-378.

Eyre, F.H., ed. 1980. Forest Cover Types of the United States and Canada. Society of American Foresters, Washington, D.C. 148 pp.

Farjon, A. 1990. Pinaceae: Drawings and Descriptions of the Genera *Abies, Cedrus, Pseudolarix, Keteleeria, Nothotsuga, Tsuga, Cathaya, Pseudotsuga, Larix* and *Picea*. Regnum Vegetabile 121. Koeltz Scientific Books, Königstein, Germany. 330 pp.

Fennessy, J., C. O'Reilly, C.P. Harper and D. Thompson. 2000. The morphology and seasonal changes in cold hardiness, dormancy intensity and root growth potential of rooted cuttings of Sitka spruce. *Forestry* 73: 489-497.

Fernando, D.D., M.D. Lazzaro and J.N. Owens. 2005. Growth and development of conifer pollen tubes. *Sexual Plant Reproduction* 18: 149-162.

FGCBC. 2001. Forest Genetics Council Business Plan 2001-2002. Forest Genetics Council of British Columbia [FGCBC], Vancouver, B.C.

Fletcher, A., Y. Birot, and S.G. Reck. 1981. Douglas-fir Case Study. Field inspection. EEC DG VI Internal Report. 32 pp.

Fletcher, A., and J.C. Bastien. 1988. Douglas-fir Seed Sources Field Inspection: Southern Washington – Northern Oregon. EEC DG VI Internal Report. 12 pp.

Fletcher, A., and J.C. Bastien. 1989. Douglas-fir seed sources field inspection: Southern Oregon – Northern California. EEC DG VI Internal Report. 11 pp.

Flous, F. 1934a. Deux espèces nouvelles de *Pseudotsuga* américains. *Bulletin de la Société d'Histoire Naturelle de Toulouse* 66: 211-224.

Flous, F. 1934b. Diagnoses d'espèces et variétés nouvelles de *Pseudotsuga* américains. *Bulletin de la Société d'Histoire Naturelle de Toulouse* 66: 329-346.

Franklin, J.F., and C.T. Dyrness. 1973. Natural Vegetation of Oregon and Washington. USDA Forest Service General Technical Report PNW-8. Pacific Northwest Region, Portland, Oregon. 417 pp.

Fu, L., N. Li, T.S. Elias and R.R. Mill. 1999. *Pseudotsuga* Carrière. Pp. 37-39 *in* Flora of China Editorial Committee, Flora of China, Vol. 4. Science Press, Beijing and Missouri Botanical Garden Press, St. Louis.

Fulé, P.Z., and W.W. Covington. 1999. Fire regime changes in La Michilía Biosphere Reserve, Durango, Mexico. *Conservation Biology* 13: 640-652.

Furniss, R.L., and V.M. Carolin. 1977. Western Forest Insects. USDA Forest Service Miscellaneous Publication 1339, Washington, D.C. 654 pp.

Gadgil, C.J., C. Zhang and W.-S. Hu. 1998. Morphological analysis of the developmental stages of Douglas-fir somatic embryos. *Molecular Biology of the Cell* 9 (Abstracts): 607. American Society for Cell Biology 38th Annual Meeting, San Francisco, California.

Garman, E.H. 1955. Regeneration Problems and their Silvicultural Significance in the Coastal Forests of British Columbia. Technical Publication T41, British Columbia Forest Service, Department of Lands and Forests, Victoria, B.C. 67 pp.

Gause, G.W. 1966. Silvical Characteristics of Bigcone Douglas-fir (*Pseudotsuga macrocarpa* [Vasey] Mayr). USDA Forest Service Research Paper PSW-39. Pacific Southwest Forest and Range Experiment Station, Berkeley, California.10 pp.

Gelbart, G., and P. von Aderkas. 2002. Ovular secretions as part of pollination mechanisms in conifers. *Annals of Forest Science* 59: 345-357.

Göhre, K., ed. 1958. Die Douglasie und ihr Holz. Akademie Verlag, Berlin. 595 pp.

González-Elizondo, M., E. Jurado, J. Návar, M.S. González-Elizondo, J. Villanueva, O. Aguirre and J. Jiménez. 2005. Tree-rings and climate relationships for Douglas-fir chronologies from the Sierra Madre Occidental, Mexico: A 1681-2001 rain reconstruction. *Forest Ecology and Management* 213: 39-53.

Gossner, M., and U. Simon. 2002. Introduced Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) affects community structure of tree-crown dwelling beetles in a managed European forest. *In* I. Kowarik and U. Starfinger, eds., Biologische Invasionen: Herausforderung zum Handeln? *Neobiota* 1: 167-179.

Griffin, A.R., and K.K. Ching. 1977. Geographic variation in Douglas-fir from the coastal ranges of California. I. Seed, seedling growth and hardiness characteristics. *Silvae Genetica* 26: 149-157.

Haddock, P.G., J. Walters and A. Kozak. 1967. Growth of Coastal and Interior Provenances of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) at Vancouver and Haney in British Columbia. University of British Columbia, Faculty of Forestry Research Paper 79.

Hamann, A., S.N. Aitken and A.D. Yanchuk. 2004. Cataloguing *in situ* protection of genetic resources for major commercial forest trees in British Columbia. *Forest Ecology and Management* 197: 295-305.

Hawksworth, F.G., and D. Wiens. 1996. Dwarf Mistletoes: Biology, Pathology, and Systematics. USDA Agriculture Handbook 709, Washington, D.C. 410 pp.

Hedlin, A.F., H.O. Yates III, D.C. Tovar, B.H. Ebel, T.W. Koerber and E.P. Merkel. 1980. Cone and Seed Insects of North American Conifers. Environment Canada, Canadian Forestry Service, Ottawa. 122 pp.

Heilman, P.E., H.W. Anderson and D.M. Baumgartner, eds. 1979. Forest Soils of the Douglas-fir Region. Washington State University, Cooperative Extension Services, Pullman, Washington. 298 pp.

Heois, B. 1994. Variabilité juvénile chez *Pseudotsuga menziesii* (Mirb.) Franco – contribution à la mise au point de tests précoces. Ph.D. thesis, Institut National de la Recherche Agronomique, Paris. Grignon, France.

Hepting, G.H. 1971. Diseases of Forest and Shade Trees of the United States. USDA Agriculture Handbook 386, Washington, D.C. 658 pp.

Hermann, R.K. 1985. The Genus *Pseudotsuga*: Ancestral History and Past Distribution. Forest Research Laboratory Special Publication 2b, College of Forestry, Oregon State University, Corvallis. 32 pp.

Hermann, R.K. 1987. North American tree species in Europe. *Journal of Forestry* 85(12): 27-32.

Hermann, R.K., and K.K. Ching. 1975. Bibliography of Douglas-fir Provenance Studies. Forest Research Laboratory Research Paper 25, School of Forestry, Oregon State University, Corvallis. 30 pp.

Hermann, R.K., and D.P. Lavender. 1990. *Pseudotsuga menziesii* (Mirb.) Franco. Pp. 527-540 *in* R.M. Burns and B.H. Honkala, tech. coords., Silvics of North America, Vol. 1. USDA Agriculture Handbook 654, Washington, D.C.

Hermann, R.K., and D.P. Lavender. 1999. Douglas-fir planted forests. *New Forests* 17: 53-70.

Hizume, M., and K. Kondo. 1992. Fluorescent chromosome banding in five taxa of *Pseudotsuga*, Pinaceae. *La Kromosomo, Series 2*, 66: 2257-2268.

Horton, T.R., T.D. Bruns and V.T. Parker. 1999. Ectomycorrhizal fungi associated with *Arctostaphylos* contribute to *Pseudotsuga menziesii* establishment. *Canadian Journal of Botany* 77: 93-102.

Howe, G.T., S.N. Aitken, D.B. Neale, K.D. Jermstad, N.C. Wheeler and T.H.H. Chen. 2003. From genotype to phenotype: Unravelling the complexities of cold adaptation in forest trees. *Canadian Journal of Botany* 81: 1247-1266.

Irgens-Möller, H. 1968. Geographical variation in growth patterns of Douglas-fir. *Silvae Genetica* 17: 106-110.

Isaac, L.A. 1943. Reproductive Habits of Douglas-fir. Charles Lathrop Pack Forestry Foundation, Washington, D.C. 107 pp.

Ishii, H., and E.D. Ford. 2001. The role of epicormic shoot production in maintaining foliage in old *Pseudotsuga menziesii* (Douglas-fir) trees. *Canadian Journal of Botany* 79: 251-264.

Jackson, S.T., and S.J. Smith. 1994. Pollen dispersal and representation on an isolated, forested plateau. *New Phytologist* 128: 181-193.

Jackson, S.T., J.L. Betancourt, M.E. Lyford, S.T. Gray and K.A. Rylander. 2005. A 40,000-year woodrat-midden record of vegetational and biogeographical dynamics in north-eastern Utah, USA. *Journal of Biogeography* 32: 1085-1106.

Jermstad, K.D., D.L. Bassoni, K.S. Jech, N.C. Wheeler and D.B. Neale. 2001a. Mapping of quantitative trait loci controlling adaptive traits in coastal Douglas-fir. I. Timing of vegetative bud flush. *Theoretical and Applied Genetics* 102: 1142-1151.

Jermstad, K.D., D.L. Bassoni, N.C. Wheeler, T.S. Anekonda, S.N. Aitken, W.T. Adams and D.B. Neale. 2001b. Mapping of quantitative trait loci controlling adaptive traits in coastal Douglas-fir. II. Spring and fall cold-hardiness. *Theoretical and Applied Genetics* 102: 1152-1158.

Johnson, G.R. 1997. Site-to-site genetic correlations and their implications on breeding zone size and optimum number of progeny test sites for coastal Douglas-fir. *Silvae Genetica* 46: 280-285.

Johnson, G.R., R.A. Sniezko and N.L. Mandel. 1998. Age trends in Douglas-fir genetic parameters and implications for optimum selection age. *Silvae Genetica* 46: 349-358.

Kennedy, P.G., and J.M. Diaz. 2005. The influence of seed dispersal and predation on forest encroachment into a California grassland. *Madroño* 52: 21-29.

Kleinschmit, J., and J.-C. Bastien. 1992. IUFRO's role in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) tree improvement. *Silvae Genetica* 41: 161-173.

Kleinschmit, J., J. Solvba, H. Weisgerber, H.-M. Rau and A. Franke. 1995. 22-year results of the 2nd stage IUFRO Douglas-fir provenance experiment in Germany. DF5: 1-18 *in* Evolution of Breeding Strategies for Conifers of the Pacific Northwest. Joint Meeting of IUFRO Working Parties S2.02.05 (Douglas-fir), S2.02.06 (*Pinus contorta*), S2.02.12 (Sitka spruce) and S2.02.14 (*Abies*), 1 -4 August, Limoges, France. INRA, Station d'Amélioration des Arbres Forestiers, Ardon, France.

Klinka, K., and R.E. Carter. 1990. Relationships between site index and synoptic environmental variables in immature coastal Douglas-fir stands. *Forest Science* 36: 815-830.

Klinka, K, R.E. Carter and M.C. Feller. 1990. Cutting old-growth forests in British Columbia: Consideration for forest regeneration. *Northwest Environmental Journal* 6: 221-242.

Klinka, K., J. Worrall, L. Skoda and P. Varga. 2000. The Distribution and Synopsis of Ecological and Silvical Characteristics of Tree Species of British Columbia's Forests. Canadian Cartographics Ltd., Coquitlam, B.C.

Klumpp, R.T. 1999. Untersuchungen zur Genökologie der Douglasie (*Pseudotsuga menziesii* [Mirb.] Franco). Dissertation, Fakultät für Forstwissenschaften und Waldökologie, Georg-August-Universität, Göttingen, Germany. 290 pp.

Knoerzer, D. 1999. Zur Naturverjungung der Douglasie im Schwarzwald. Inventur und Analyze von Umwelt- und Kondurrenzfaktoren sowie eine naturschutzfachliche Bewertung. Diss. Bot. 306. 283 pp. + Supplement.

Knoerzer, D., and A. Reif. 2001. Provision and strategies for the management of Douglas-fir. On controlling naturalisation effects. Pp. 55-56 *in* I. Kowarik and U. Starfinger, eds., Biological Invasions in Germany. A Challenge to Act? BfN Scripten 32, Bonn.

Krajina, V.J. 1965. Biogeoclimatic zones and biogeocoenoses of British Columbia. Ecology of Western North America 1: 1-17. University of British Columbia, Department of Botany, Vancouver.

Krajina, V.J. 1969. Ecology of forest trees in British Columbia. Ecology of Western North America 2: 1-146. University of British Columbia, Department of Botany, Vancouver.

Kranenborg, K.G., and S.M.G. de Vries. 1995. Douglas-fir provenance research in The Netherlands; 1966-67 IUFRO Series. Institute for Forestry and Nature Research (IBN-DLO) IBN Research Report 95/1, Wageningen, The Netherlands.

Kuiper, L.C., and M.P. Coutts. 1992. Spatial disposition and extension of the structural root system of Douglas-fir. *Forest Ecology and Management* 47: 111-125.

Lavender, D.P. 1984. Plant physiology and nursery environments: Interactions affecting seedling growth. Pp. 133-141 *in* M.L. Duryea and T.D. Landis, eds., Forest Nursery Manual: Production of Bare-rooted Seedlings. Martinus Nijhoff / Dr. W. Junk Publishers, Boston, Massachusetts.

Ledgard, N.J., and E.R. Langer. 1999. Wilding Prevention. Guidelines for Minimising the Risk of Unwanted Wilding Spread from New Plantings of Introduced Conifers. New Zealand Forest Research Institute, Christchurch, New Zealand.

Li, P., and W.T. Adams. 1989. Range-wide patterns of allozyme variation in Douglas-fir (*Pseudotsuga menziesii*). *Canadian Journal of Forest Research* 19: 149-161.

Li, P., and W.T. Adams. 1993. Genetic control of bud phenology in pole-size trees and seedlings of coastal Douglas-fir. *Canadian Journal of Forest Research* 23: 1043-1051.

Li, P., and W.T. Adams. 1994. Genetic variation in cambial phenology of coastal Douglas-fir. *Canadian Journal of Forest Research* 24: 1864-1870.

Lipow, S.R., K. Vance-Borland, J.B. St. Clair, J. Henderson and C. McCain. 2004. Gap analysis of conserved genetic resources for forest trees. *Conservation Biology* 18: 412-423.

Lipscomb, B. 1993. *Pseudotsuga* Carrière. Pp. 365-366 *in* Flora of North America Editorial Committee, Flora of North America, Vol. 2. Oxford University Press, New York and Oxford.

Little Jr., E.L. 1979. Checklist of United States Trees (Native and Naturalized). USDA Agriculture Handbook 541, Washington, D.C. 375 pp.

MacLaren, J.P. 1996. Environmental Effects of Planted Forests in New Zealand. Bulletin 198, New Zealand Forest Research Institute, Rotorua, New Zealand.

Magnussen, S., and A.D. Yanchuk. 1994. Time trends of predicted breeding values in selected crosses of coastal Douglas-fir in British Columbia – a methodological study. *Forest Science* 40: 663-685.

Marshall, K.A., and D.B. Neale. 1992. The inheritance of mitochondrial DNA in Douglas-fir (*Pseudotsuga menziesii*). *Canadian Journal of Forest Research* 22: 73-75.

Martínez, M. 1963. Las Pináceas Mexicanas, 3rd ed. Universidad Nacional Autónoma de México, Mexico, D.F. 401 pp.

McArdle, R.E., W.H. Meyer and D. Bruce. 1961. The Yield of Douglas-fir in the Pacific Northwest. USDA Forest Service Technical Bulletin 201. 74 pp.

McConnon, H., R.L. Knowles and L.W. Hansen. 2004. Provenance affects bark thickness in Douglas-fir. *New Zealand Journal of Forestry Science* 34: 77-86.

Meidinger, D.V., and J. Pojar, eds. 1991. Ecosystems of British Columbia. Special Report Series 6, British Columbia Ministry of Forests, Victoria, B.C. 330 pp.

Miller, R.E., P.R. Barker, C.E. Peterson and S.R. Webster. 1986. Using nitrogen fertilizer in management of coastal Douglas-fir: I. Regional trends and response. Pp. 290-303 *in* C.D. Oliver, D.P. Hanley, and J.A. Johnson, eds., Douglas-fir: Stand Management for the Future. Contribution 55, College of Forest Resources, University of Washington, Seattle.

Mitton, J.B. 1992. The dynamic mating systems of conifers. *New Forests* 6: 197-216.

Moore, J.A. 1988. Response of Douglas-fir, grand fir, and western white pine to forest fertilization. Pp. 226-230 *in* W.C. Schmidt, compiler, Future Forests of the Mountain West: A Stand Culture Symposium. USDA Forest Service General Technical Report INT-243. Intermountain Research Station, Ogden, Utah.

Munger, T.R., and W.G. Morris. 1936. Growth of Douglas-fir Trees of Known Seed Source. USDA Technical Bulletin 537.

Neale, D.B., and W.T. Adams. 1985. The mating system in natural and shelterwood stands of Douglas-fir. *Theoretical and Applied Genetics* 71: 201-207.

Neale, D.B., N.C. Wheeler and R.W. Allard. 1986. Paternal inheritance of chloroplast DNA in Douglas-fir. *Canadian Journal of Forest Research* 16: 1152-1154.

Nelson, R.J., M. Stoehr, G. Cooper, C.T. Smith and H. Mehl. 2003. High levels of chloroplast genetic variation differentiate coastal and interior Douglas-fir (*Pseudotsuga menziesii*) lineages in southern British Columbia. *Forest Genetics* 10: 153-157.

O'Brien, I.E.W., D.R. Smith, R.C. Gardner and B.G. Murray. 1996. Flow cytometric determination of genome size in *Pinus*. *Plant Science* 115: 91-99.

O'Neill, G.A., S.N. Aitken and W.T. Adams. 2001. Quantitative genetics of spring and fall cold hardiness in seedlings from two Oregon populations of coastal Douglas-fir. *Forest Ecology and Management* 149: 305-318.

Orlić, S., and M. Ocvirek. 1995. IUFRO Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) provenances experiment in Croatia. DF5: 1-9 *in* Evolution of Breeding Strategies for Conifers of the Pacific Northwest. Proceedings of the Joint Meeting of IUFRO Working Parties S2.02.05 (Douglas-fir), S2.02.06 (*Pinus contorta*), S2.02.12 (Sitka spruce) and S2.02.14 (*Abies*), 1 -4 August, Limoges, France. INRA, Station d'Amélioration des Arbres Forestiers, Ardon, France.

Orr-Ewing, A.L. 1966a. E.P. 513: Inter- and intraspecific crosses within the genus *Pseudotsuga*. British Columbia Forest Service, Forest Research Review 1966: 13-15.

Orr-Ewing, A.L. 1966b. Inter- and intraspecific crossing in Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco. *Silvae Genetica* 15: 121-126.

Orr-Ewing, A.L. 1971. The Asiatic *Pseudotsugae*. British Columbia Forest Service, Forest Research Review 1971: 95-96.

Orr-Ewing, A.L. 1975. Experimental Project 513. British Columbia Forest Service, Forest Research Review 1975: 40.

Orr-Ewing, A.L., A.R. Fraser and I. Karlsson. 1972. Interracial Crosses with Douglas-fir: Early Field Results. British Columbia Forest Service, Research Notes 55. 33 pp.

Owens, J.N. 1973. The Reproductive Cycle of Douglas-fir. Canadian Forestry Service Pacific Forest Research Centre Report BC-P-8. 23 pp.

Owens, J.N., S.J. Simpson and M. Molder. 1981. The pollination mechanism and the optimal time of pollination in Douglas-fir (*Pseudotsuga menziesii*). *Canadian Journal of Forest Research* 11: 36-50.

Owens, J.N., S.J. Morris and G.L. Catalano. 1994. How the pollination mechanism, prezygotic and postzygotic events affect seed production in *Larix occidentalis*. *Canadian Journal of Forest Research* 24: 2222-2234.

Owston, P.W., and W.I. Stein. 1974. *Pseudotsuga* Carr., Douglas-fir. Pp. 674-683 *in* C.S. Schopmeyer, tech. coord., Seeds of Woody Plants in the United States. USDA Agriculture Handbook 450, Washington, D.C.

Peterson, G.W., and R.S. Smith Jr., tech. coords. 1975. Forest Nursery Diseases in the United States. USDA Agriculture Handbook 470, Washington, D.C. 125 pp.

Pfister, R.D., B.L. Kovalchik, S.F. Arno and R.C. Presby. 1977. Forest Habitat Types of Montana. USDA Forest Service General Technical Report INT-34. Intermountain Research Station, Ogden, Utah. 174 pp.

Ponoy, B., Y.P. Hong, J. Woods, B. Jaquish and J.E. Carlson. 1994. Chloroplast DNA diversity of Douglas-fir in British Columbia. *Canadian Journal of Forest Research* 24: 1824-1834.

Poulis, B.A.D., S.J.B. O'Leary, J.D. Haddow and P. von Aderkas. 2005. Identification of proteins present in the Douglas-fir ovular secretion: An insight into conifer pollen selection and development. *International Journal of Plant Sciences* 166: 733-739.

Prat, D. 1995. Mating system in a clonal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seed orchard. II. Effective pollen dispersal. *Annales des Sciences Forestières* 52: 213-222.

Prat, D., and S. Arnal. 1994. Allozyme variation and mating system in three artificial stands of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) planted in Europe. *Silvae Genetica* 43: 199-206.

Prat, D., and T. Caquelard. 1995. Mating system in a clonal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seed orchard. I. Gene diversity and structure. *Annales des Sciences Forestières* 52: 201-211.

Randall, W.K. 1996. Forest Tree Seed Zones for Western Oregon. Oregon Department of Forestry, Salem, Oregon. 82 pp.

Randall, W.K., and P. Berrang. 2002. Washington Tree Seed Transfer Zones. Washington Department of Natural Resources, Olympia, Washington. 63 pp.

Rehfeldt, G.E. 1974. Local differentiation of populations of Rocky Mountain Douglas-fir. *Canadian Journal of Forest Research* 4: 399-406.

Rehfeldt, G.E. 1979a. Patterns of First-year Growth in Populations of Douglas-fir (*Pseudotsuga menziesii* var. *glauca*). USDA Forest Service Research Note INT-255. Intermountain Research Station, Ogden, Utah. 8 pp.

Rehfeldt, G.E. 1979b. Variation in Cold Hardiness Among Populations of *Pseudotsuga menziesii* var. *glauca*. USDA Forest Service Research Paper INT-233. Intermountain Research Station, Ogden, Utah. 12 pp.

Rehfeldt, G.E. 1983. Ecological adaptations in Douglas-fir (*Pseudotsuga menziesii* var. *glauca*). III. Central Idaho. *Canadian Journal of Forest Research* 13: 626-632.

Reyes-Hernández, V.J., J.J. Vargas-Hernández, J. López-Upton and H. Vaquera-Huerta. 2005. Variación morfológica y anatómica en poblaciones mexicanas de *Pseudotsuga* (Pinaceae). *Acta Botanica Mexicana* 70: 47-67.

Ritchie, G.A., S.D. Duke and R. Timmis. 1994. Maturation in Douglas-fir. 2. Maturation characteristics of genetically matched Douglas-fir seedlings, rooted cuttings and tissue-culture plantlets during and after 5 years of field growth. *Tree Physiology* 14: 1261-1275.

Ritchie, G.A., J.W. Keeley and P.A. Ward. 1997. Effects of shade and root confinement on the expression of plagiotropic growth in juvenile-origin Douglas-fir rooted cuttings. *Canadian Journal of Forest Research* 27: 1142-1145.

Rowe, K.E., and K.K. Ching. 1973. Provenance study of Douglas-fir in the Pacific Northwest region. *Silvae Genetica* 22: 115-119.

Ryker, R.A. 1975. Survey of Factors Affecting Regeneration of Rocky Mountain Douglas-fir. USDA Forest Service Research Paper INT-174. Intermountain Research Station, Ogden, Utah. 19 pp.

Schermann, N., W.T. Adams, S.N. Aitken and J.C. Bastien. 1997. Genetic parameters of stem form traits in a 9-year-old coastal Douglas-fir progeny test in Washington. *Silvae Genetica* 46: 166-170.

Schmidt, R.G. 1957. The Silvics and Plant Geography of the Genus *Abies* in the Coastal Forests of British Columbia. Technical Publication T46, British Columbia Forest Service, Department of Lands and Forests, Victoria, B.C. 31 pp.

Schmidt, W.C. 1969. Seedbed Treatments Influence Seedling Development in Western Larch Forests. USDA Forest Service Research Note INT-93. Intermountain Research Station, Ogden, Utah. 7 pp.

Schorn, H.E., and A. Thompson. 1998. The genus *Pseudotsuga*: A revision of the fossil record and inferred paleo-biogeographical and migrational patterns. Abstract 27 *in* UCMP 75th/125th Anniversary: Integrative Paleontology and the Future, February 28, 1998. Museum of Paleontology, University of California, Berkeley.

Schubert, G.H., and R.S. Adams. 1971. Reforestation Practices for Conifers in California. California Division of Forestry, Sacramento, California. 359 pp.

Silen, R.R. 1963. Effects of altitude on factors of pollen contamination of Douglas-fir seed orchards. *Journal of Forestry* 61: 281-283.

Silen, R.R. 1978. Genetics of Douglas-fir. USDA Forest Service Research Paper WO-35, Washington, D.C. 34 pp.

Silen, R.R., and J.G. Wheat. 1979. Progressive tree improvement program in coastal Douglas-fir. *Journal of Forestry* 77: 78-83.

Simberloff, D., M.A. Relva and M. Nuñez. 2003. Introduced species and management of a *Nothofagus/ Austrocedrus* forest. *Environmental Management* 31: 263-275.

Slavov, G.T., G.T. Howe and W.T. Adams. 2005. Pollen contamination and mating patterns in a Douglas-fir seed orchard as measured by simple sequence repeat markers. *Canadian Journal of Forest Research* 35: 1592-1603.

Sorensen, F.C. 1969. Embryonic genetic load in coastal Douglas-fir, *Pseudotsuga menziesii* var. *menziesii*. *American Naturalist* 103: 389-398.

Sorensen, F.C. 1997. Effects of sib mating and wind pollination on nursery seedling size, growth components, and phenology of Douglas-fir seed orchard progenies. *Canadian Journal of Forest Research* 27: 557-566.

Sorensen, F.C., and D.W. Cress. 1994. Effects of sib mating on cone and seed traits in coastal Douglas-fir. *Silvae Genetica* 43: 338-345.

St. Clair, J.B., and W.T. Adams. 1991. Relative family performance and variance structure of open-pollinated Douglas-fir seedlings grown in three competitive environments. *Theoretical and Applied Genetics* 81: 541-550.

St. Clair, J.B., N.L. Mandel and K.W. Vance-Borland. 2005. Genecology of Douglas-fir in western Oregon and Washington. *Annals of Botany* 96: 1199-1214.

Stein, W.I., and P.W. Owston. 2002. *Pseudotsuga* Carr., Douglas-fir. 32 pp., 4 figs. *in* F.T. Bonner, tech. coord., and R.G. Nisley, ed., Woody Plant Seed Manual. USDA Forest Service, Washington, D.C. http://www.nsl.fs.fed.us/wpsm/Pseudotsuga.pdf.

Steiner, K.C. 1979. Variation in bud-burst timing among populations of interior Douglas-fir. *Silvae Genetica* 28: 76-79.

Stimm, B., and P.H. Dong. 2001. The Kaiserslautern Douglas-fir provenance trial after nine decades of observation. *Forstwissenschaftliches Centralblatt* 120: 173-186.

Stoehr, M.U., B.L. Orvar, T.M. Vo, J.R. Gawley, J.E. Webber and C.H. Newton. 1998. Application of a chloroplast DNA marker in seed orchard management evaluations of Douglas-fir. *Canadian Journal of Forest Research* 28: 187-195.

Stoehr, M.U., J.E. Webber and R.A. Painter. 1994. Pollen contamination effects on progeny from an off-site Douglas-fir seed orchard. *Canadian Journal of Forest Research* 24: 2113-2117.

Stonecypher, R.W., R.F. Piesch, G.G. Helland, J.G. Chapman and J.H. Reno. 1996. Results from Genetic Tests of Selected Parents of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in an Applied Tree Improvement Program. *Forest Science* 42(2), Suppl.; *Forest Science Monograph* 32. 35 pp.

Sullivan, T.P., and D.S.Sullivan. 1984. Operational Direct Seeding of Douglas-fir and Lodgepole Pine with Alternative Foods in British Columbia. British Columbia Ministry of Forests Research Note 97. Victoria, B.C. 16 pp.

Sutherland, J.R., and E. van Eerden. 1980. Diseases and Insect Pests in British Columbia Forest Nurseries. Joint Report 12, British Columbia Ministry of Forests & Canadian Forestry Service, Victoria, B.C. 55 pp.

Sweet, G.B. 1964. Provenance differences in Pacific Coast Douglas-fir. *Silvae Genetica* 14: 46-56.

Taber, R.P., C. Zhang and W.S. Hu. 1998. Kinetics of Douglas-fir (*Pseudotsuga menziesii*) somatic embryo development. *Canadian Journal of Botany* 76: 863-871.

Takaso, T., and J.N. Owens. 1995. Ovulate cone morphology and pollination in *Pseudotsuga* and *Cedrus*. *International Journal of Plant Sciences* 156: 630-639.

Thompson, A., and H.E. Schorn. 1998. Mechanisms of migration in Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco]: A model. Abstract 36 *in* UCMP 75th/125th Anniversary: Integrative Paleontology and the Future, February 28, 1998. Museum of Paleontology, University of California, Berkeley.

Timmis, R., J. Flewelling and C. Talber. 1994. Frost injury prediction model for Douglas-fir seedlings in the Pacific Northwest. *Tree Physiology* 14: 855-869.

Tsukada, M. 1982. *Pseudotsuga menziesii* (Mirb.) Franco: Its pollen dispersal and Late Quaternary history in the Pacific Northwest. *Japanese Journal of Ecology* 32: 159-187.

Van Pelt, R. 2001. Forest Giants of the Pacific Coast. University of Washington Press, Seattle. 200 pp.

Vargas-Hernández, J., and W.T. Adams. 1991. Genetic variation of wood density components in young coastal Douglas-fir – implications for tree breeding. *Canadian Journal of Forest Research* 21: 1801-1807.

Vargas-Hernández, J.J., J. López-Upton, V.J. Reyes-Hernández, F.A. Domínguez-Álvarez and M. Mapula-Larreta. 2004. Natural populations of Douglas-fir in Mexico: Current status and needs for conservation. Pp. 26-36 in J. Beaulieu, ed., Silviculture and the Conservation of Genetic Resources for Sustainable Forest Management. Proceedings of the Symposium of the North American Forest Commission, Forest Genetic Resources and Silviculture Working Groups, and the International Union of Forest Research Organizations, Quebec City, Canada, September 21, 2003. Information Report LAU-X-128, Canadian Forest Service, Laurentian Forestry Centre, Sainte-Foy, Quebec.

Vega, G., A.D. Ruiz, V. García and J.L. Jenkinson. 1995. Pacific Douglas-fir: 15-year performance in Ochagavia common-garden test, the Pyrenees, Navarra, Spain. DF3 *in* Evolution of Breeding Strategies for Conifers of the Pacific Northwest. Proceedings of the Joint Meeting of IUFRO Working Parties S2.02.05 (Douglas-fir),

S2.02.06 (*Pinus contorta*), S2.02.12 (Sitka spruce) and S2.02.14 (*Abies*), 1-4 August, Limoges, France. INRA, Station d'Amélioration des Arbres Forestiers, Ardon, France.

Viard, F., Y.A. El-Kassaby and K. Ritland. 2001. Diversity and genetic structure in populations of *Pseudotsuga menziesii* (Pinaceae) at chloroplast microsatellite loci. *Genome* 44: 336-344.

von Aderkas, P., and C. Leary. 1999. Micropylar exudates in Douglas-fir – timing and volume of production. *Sexual Plant Reproduction* 11: 354-356.

von Aderkas, P., G. Rouault, R. Wagner, S. Chiwocha and A. Roques. 2005a. Multinucleate storage cells in Douglas-fir (*Pseudotsuga menziesii* (Mirbel) Franco) and the effect of seed parasitism by the chalcid *Megastigmus spermotrophus* Wachtl. *Heredity* 94: 616-622.

von Aderkas, P., G. Rouault, R. Wagner, R. Rhor and A. Roques. 2005b. Seed parasitism redirects ovule development in Douglas-fir. *Proceedings of the Royal Society B: Biological Sciences* 272: 1491-1496.

von Rudloff, E. 1972. Chemosystematic studies in the genus *Pseudotsuga*. 1. Leaf oil analysis of the coastal and Rocky Mountain varieties of the Douglas-fir. *Canadian Journal of Botany* 50: 1025-1040.

Wang, X.-Q., D.C. Tank and T. Sang. 2000. Phylogeny and divergence times in Pinaceae: Evidence from three genomes. *Molecular Biology and Evolution* 17: 773-781.

Webber, J.E., and R.A. Painter. 1996. Douglas-fir Pollen Management Manual, 2nd ed. British Columbia Ministry of Forests, Research Branch, Working Paper 02/1996. Victoria, B.C. 91 pp.

White, T.L., and K.K. Ching. 1985. Provenance study of Douglas-fir in the Pacific Northwest region. IV. Field performance at age 25 years. *Silvae Genetica* 34: 84-90.

Winter, L.E., L.B. Brubaker, J.F. Franklin, E.A. Miller and D.Q. DeWitt. 2002a. Canopy disturbances over the five-century lifetime of an old-growth Douglas-fir stand in the Pacific Northwest. *Canadian Journal of Forest Research* 32: 1057-1070.

Winter, L.E., L.B. Brubaker, J.F. Franklin, E.A. Miller and D.Q. DeWitt. 2002b. Initiation of an old-growth Douglas-fir stand in the Pacific Northwest: A reconstruction from tree-ring records. *Canadian Journal of Forest Research* 32: 1039-1056.

Woods, J.H., D. Kolotelo and A.D. Yanchuk. 1995. Early selection of coastal Douglas-fir in a farm-field test environment. *Silvae Genetica* 44: 178-186.

Yanchuk, A.D. 1996. General and specific combining ability from disconnected partial diallels of coastal Douglas-fir. *Silvae Genetica* 45: 37-45.

Zhang, C., R. Timmis and W.S. Hu. 1999. A neural network based pattern recognition system for somatic embryos of Douglas-fir. *Plant Cell, Tissue and Organ Culture* 56: 25-35.

Section 5.
Lodgepole pine (*Pinus contorta*)

Preamble:

The following text applies principally to lodgepole pine (*Pinus contorta* Dougl. *ex*. Loud.) in the most important part of its range; namely central and southern British Columbia, western Alberta, eastern Washington, eastern Oregon, Idaho, Montana, Wyoming, northern Colorado, and northern Utah. It also discusses use of lodgepole pine as an exotic.

1. Taxonomy

The genus *Pinus* L. (in the family Pinaceae) originated in the early to mid-Mesozoic about 180 million years ago, prior to the continental separation in the Laurasian region that became eastern North America and western Europe (Burdon, 2002). Some 150 million years before the present (BP), *Pinus* subdivided into hard pines (subgenus *Pinus*) and soft pines (subgenus *Strobus*). Rapid evolution, speciation, and migration occurred during the Tertiary prior to cooling climatic conditions at its end (Mirov and Hasbrouck, 1976). Lodgepole pine (*Pinus contorta* Dougl. *ex*. Loud.) and its close relative jack pine (*P. banksiana* Lamb.) might have evolved from a common progenitor into a western and a northern species during cooling in the late Tertiary (Pliocene), or may not have diverged until the Pleistocene (Critchfield, 1984) — Dancik and Yeh (1983) estimated that they diverged between 485,000 and 565,000 BP.

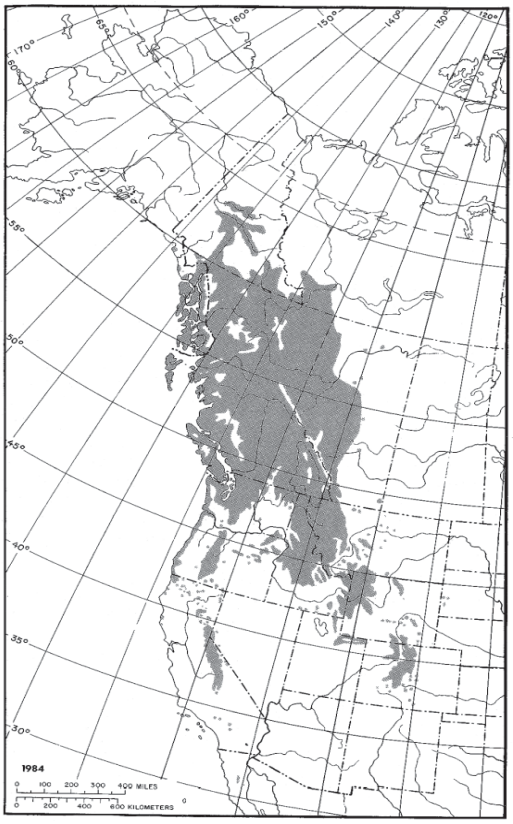
Lodgepole pine is a western North American 2-needled pine of the subgenus *Pinus* (much resin, close-grained wood, sheath of leaf cluster persistent, two vascular bundles in each needle), section *Pinus*, subsection *Contortae*, along with the North American species *P. banksiana*, *P. virginiana* and *P. clausa* (Little and Critchfield, 1969). The stiff usually twisted needles are 2.5-7.6 cm long; cones are near branch tips, each cone scale with a short spine. Lodgepole pine has evolved into several highly differentiated but interfertile geographic races that differ morphologically and ecologically. Four subspecies (Critchfield, 1957), also referred to as varieties (Little, 1979), are recognized:

- *Pinus contorta* subsp. *contorta* – a coastal, somewhat crooked shorter race, known as shore pine, coast pine, or beach pine;
- *Pinus contorta* subsp. *bolanderi* (Parl.) Critchf. – a closed-cone (serotinous) stunted local form in north-western California (Mendocino County, endemic on podzol soils), which is called Bolander pine, and by some considered a synonym under *P. contorta* subsp. *contorta* (Aitken and Libby, 1994; Kral, 1993);
- *Pinus contorta* subsp. *murrayana* (Grev. & Balf.) Critchf. – a non-serotinous, far western montane race in that Cascades (Oregon) to Mexico but primarily in the Sierra Nevada of California, which is called Sierra lodgepole pine or sometimes tamarack pine; and
- *Pinus contorta* subsp. *latifolia* (Engelm. in S. Wats.) Critchf.– the extensively distributed continental interior race, which is often straight and tall, and referred to simply as lodgepole pine or sometimes as Rocky Mountain lodgepole pine or black pine.

2. Natural distribution

Lodgepole pine is a commonly occurring Western North American (and marginally central North American) species with a wide latitudinal and elevational range (Wheeler and Guries, 1982a; Klinka *et al.*, 2000) (Figure 1). It grows throughout the Rocky Mountain and Pacific regions, with a range extending from approximately 31°N in Baja California north to around 64°N in the Yukon Territory, and from the Pacific Ocean east to South Dakota. Although subsp. *contorta* and *bolanderi* are not found above 610 m, the interior subsp. *latifolia* and *murrayana* together span from 490 to 3,660 m (Little, 1979). Forests dominated by lodgepole pine cover approximately 26 million ha in North America, with the majority of this area is in Canada (20 million ha) (Lotan and Critchfield, 1990; MacDonald and Cwynar, 1985; Griffin and Critchfield, 1976).

Figure 1 The native range of lodgepole pine



Source: from Lotan and Critchfield, 1990

3. Reproductive biology

3.1. Reproductive development

Lodgepole pine is monoecious, with male and female strobili ("flowers") usually borne separately on the same tree. Female strobili are usually at the apical end of main branches in the upper crown, while pollen strobili originate in the lower crown. Female strobili are reddish-purple and develop in whorls of two to five. Pollen cones are pale yellow to yellowish- orange and occur in crowded clusters at the base of new shoots (Lotan and Critchfield, 1990).

Buds differentiate into male, female or vegetative the summer prior to strobili emergence. Pollen strobili emerge in spring and generally mature from mid-May to mid-July (Satterlund, 1975; Critchfield, 1980). The timing of pollen maturation and female receptivity appears to be related to elevation and climate. Pollen dispersal is *via* wind. Pollen is drawn into the micropyle in a pollination drop. Fertilization occurs nearly one year after pollination, then cones complete development and mature in August, September, or October of that year (Owens and Molder, 1994). Inland and high elevation stands mature earlier than coastal or low elevation stands. At maturity, cones change from purple-green to light brown in colour (Schopmeyer, 1974).

3.2. Mating system and gene flow

The mating system of lodgepole pine is outcrossing, with both single and multilocus estimates of outcrossing rate (t) based on allozymes approaching one (Yeh and Layton, 1979; Epperson and Allard, 1984). Selfing estimates based on phenotypic frequencies of progeny of open-pollinated trees carrying recessive, mutant markers indicated a selfing rate of 4.3% in the upper crown and 9.6% in the middle crown (Sorensen and Adams, 1993).

Lodgepole pine, like all pines, is wind pollinated and has pollen grains with two air sacs, facilitating long-distance dispersal, thus gene flow is generally thought to be high for this species (Yang and Yeh, 1995). Studies using paternal chloroplast and maternal mitochondrial genetic markers indicate that gene flow is higher *via* pollen than seed, as would be expected (Dong and Wagner, 1994). Gene flow is higher among central, continuous populations than among disjunct, or marginal populations (Yeh and Layton, 1979; cf. Delcourt and Delcourt, 1991; Fazekas and Yeh, 2001), and appears to be highest for subsp. *latifolia*, intermediate for subsp. *contorta*, and lowest for subsp. *murrayana*, based on indirect estimates from population differentiation statistics (Yang and Yeh, 1993).

3.3. Seed production

Lodgepole pine is a highly fecund species, and trees commonly start producing viable seed at 5 to 10 years, with the percentage of germination as high as that of mature trees. Female and male strobili have been observed on two year-old seedlings. This high and early fecundity contributes to lodgepole pine's ability to naturalize and become invasive in some foreign environments such as New Zealand (Ledgard, 1993; Richardson and Higgins, 1998). Good cone crops usually occur at 1- to 3-year intervals, with light crops in between. Only squirrels (*Sciuridae*) and coreid insects are significant seed predators (Lotan and Critchfield, 1990).

Lodgepole pine seeds are relatively small compared to other species of pine. The number of cleaned seed per kg ranges between 200,000 and 300,000, depending on subspecies (Lotan and Perry, 1983). Seed weights vary considerably and increase from north to south. The number of cleaned seeds averaging 207,000 per kg for subsp. *latifolia*, 258,000 per kg for subsp. *murrayana* and 298,000 per kg for subsp. *contorta* (Critchfield, 1980). Filled seed per cone can range from 5 to 45, and averages around 20 (Critchfield, 1980).

Individual dominant and codominant trees can produce from a few hundred to a few thousand cones per tree (Lotan, 1975). Annual production for subsp. *latifolia* may run from 173,000 to 790,000 seeds per hectare with half to one-third available for annual seedfall and the remaining held viable in closed serotinous cones (Fowells, 1965; Critchfield, 1980). In New Zealand, subsp. *latifolia*, *murrayana* and *contorta* have all been introduced, but subsp. *contorta* produced seed earlier and more prolifically than the other subspecies, contributing to its role as a "noxious weed" (Ledgard, 1993). Cones are persistent and the majority are serotinous for mature trees in more northern areas of subsp. *latifolia*. Closed cones can accumulate for decades. Juvenile subsp. *latifolia* produce mostly non-serotinous cones. In Oregon, where cones are primarily non-serotinous, seedfall ranges from about 35,000 to over 1.2 million per ha

(Dahms, 1963). In the serotinous cones of subsp. *latifolia*, stored seeds are in the millions per hectare and the number of seeds stored is probably 10 times that of seeds produced annually (Lotan, 1975).

The serotinous cone habit varies over a wide range of geographic scales (Lotan, 1975). While this habit is typical of most of the range of subsp. *latifolia*, trees of this subspecies in eastern Oregon are mostly non-serotinous (Lotan and Critchfield, 1990). Serotinous cones are also rare in coastal populations (subsp. *contorta*), and absent in the Sierra Nevada and southern California and Baja California populations (subsp. *murrayana*), but are found in Bolander pine (subsp. *bolanderi*) (Critchfield, 1980). The scales of serotinous cones cannot flex open due to a resinous bond. These bonds break on exposure to temperatures between 45° and 60°C (Perry and Lotan, 1977). After resinous bonds break, cone scales can flex open hygroscopically and release seeds. Closed cones at or near the soil surface (less than 30 cm depth) are subjected to insolation temperatures sufficient to break resinous bonds, and may provide seed for natural regeneration in harvested areas. The potential for weedy invasiveness of lodgepole pine as an exotic may be affected by cone serotiny and the presence or absence of fire (Ledgard, 1993; Richardson and Higgins, 1998). In northern Europe, subsp. *latifolia* originating from the northern portion of the range with predominantly serotinous cones has not proven weedy or invasive, with only limited naturalization despite wide scale planting, whereas in New Zealand subsp. *contorta* has spread rapidly from seed dispersed from cones opening at maturity (Ledgard, 1993).

Lodgepole pine is a fire-maintained, subclimax species. Its ability to regenerate to extremely high densities and exclude other species can be attributed to the closed cone habit. Millions of seeds per hectare held in reserve for many years are readily available to germinate. In addition to opening cones, fire prepares an ideal seedbed. It appears that fire is a strong agent of natural selection favouring serotinous cones (Perry and Lotan, 1979). It is possible that the serotinous cone habit could be lost if landraces develop where subsp. *latifolia* has been introduced as an exotic in environments with low fire frequency and intensity, e.g., northern Europe.

Seeds remain viable in serotinous cones for years. Viability can be maintained as long as cones or seeds are not on the ground. Once cones are on the ground, they open. Damping-off fungi may infect the seed, rodents may feed on the seeds, or germination may occur. Seeds are not stored in soil seedbanks (Lotan and Critchfield, 1990).

3.4. Natural regeneration

Lodgepole pine is best maintained using even-aged silvicultural systems (Lotan, 1975). Clear cutting followed by either planting or natural regeneration is common. Although success of natural regeneration is high, planting allows for initial stocking control and genetic improvement. Natural regeneration requires an adequate seed source, an appropriate seedbed, and suitable microsites to succeed.

For non-serotinous cones that disperse seeds from standing trees, the density of seedfall 20 m from the timber edge is only 10 to 30% of that at the stand edge in the Rocky Mountains (Lotan and Perry, 1983). Dispersal of sufficient seed to adequately restock an area often only occurs within 60 m of the seed source (Dahms and Barrett, 1975; Lotan, 1975). Prevailing winds, thermal effects, or scudding on snow or ice may disperse seeds far beyond these distances, however. For example, in New Zealand the furthest documented seedling establishment from a seed source is 30 km (Ledgard, 1993). The annual seedfall from the non-serotinous cones of initial colonizers helps to fully occupy sites. Seedfall can also restock stands following relatively minor disturbances in a stand and maintain lodgepole pine in mixed stands. There are usually some trees with non-serotinous cones in most stands. Most seeds in mature non-serotinous cones are released in fall and winter (Fowells, 1965).

When stands are harvested and the resulting slash contains large numbers of serotinous cones, appropriate slash treatments can result in sufficient seed dispersal for natural regeneration. If cones become detached from the slash, they can open with normal summer soil surface temperatures (Lotan,

1964). The seed supply will be largely destroyed if slash to be burned is piled before cones have had a chance to open (Lotan, 1975). After sufficient cones have opened, piling slash scatters seeds and helps prepare the seedbed. Most seed is released from serotinous cones near the ground during the first year. Serotinous cones that are suspended well above the ground will remain closed, and the seed they contain will remain viable for years. Broadcast burning can be used to accelerate the release of seeds from such cones or from those with limited exposure to sunlight. Some seeds will be destroyed; however, the amount will vary with fire intensity.

3.5. Vegetative reproduction

Lodgepole pine vegetatively reproduces only rarely in nature. Some natural sprouting has been observed in the Bitterroot National Forest in Montana. Branches on stumps from thinning often become leaders. Lodgepole pine is regularly grafted into seed orchards, but the success of grafting can depend on the clone (Critchfield, 1980). Juvenile lodgepole pine cuttings are relatively easy to root, but rootability varies with clone and declines with donor age (Fries and Kaya, 1997a). Seedlings can be hedged to maintain juvenility and provide cuttings (Fries and Kaya, 1997b). Many needle fascicles (short shoots) can be stimulated to produce long shoots by pinning seedlings horizontally along the soil, then the shoots produced can be cut and easily rooted (S.N. Aitken, University of British Columbia, unpublished data). Callus tissue cultures and liquid cell suspensions have been produced from seedling hypocotyl tissue, excised embryos, and actively growing shoots (Cole, 1975). There are no published reports regarding somatic embryogenesis in lodgepole pine, but other *Pinus* species have been successfully cloned in this manner so it is highly likely that this technology could be developed, providing an effective system within which transformation and regeneration of transgenic emblings could be achieved.

4. Genetics

4.1. Cytology

Pinus contorta has a haploid complement of n=12 chromosomes, like all species in the genus *Pinus* and most in the family Pinaceae (Wright, 1962). Aneuploids and polyploids are unknown in this species. The inheritance of cytoplasmic organelles has been studied using genetic markers, and like other species in the Pinaceae, mitochondria are inherited largely maternally with some paternal leakage, whereas chloroplasts are inherited paternally (Wagner *et al.*, 1991a; Dong *et al.*, 1992; Dong and Wagner, 1994).

4.2. Genetic variation

4.2.1. Population-level variability

The results of numerous allozyme studies of among and within subspecies and population variation are compiled in Table 1. Wheeler and Guries (1982b) compared seed and cone morphology with allozyme frequencies and found that while 38% of the variation in morphology was due to differences among subspecies, and 19% due to variation among populations within subspecies, for allozymes just 3% of the variation was among subspecies and 6% among populations within subspecies. The strong morphological differences among subspecies support Critchfield's 1957 taxonomic treatment (Wheeler and Guries, 1982b; Newman and Jancey, 1983). Marginal (disjunct or peripheral) populations show a higher degree of population differentiation than core populations, presumably due to reduced gene flow (Fazekas and Yeh, 2001; Yeh and Layton, 1979). Populations separated by short distances (one or two km) differ very little genetically (Knowles, 1984).

Highly variable microsatellite markers (SSR, single sequence repeats) and randomly amplified polymorphic DNA (RAPD) markers have been developed for lodgepole pine (Hicks *et al.,* 1998). Expected heterozygosities for these markers range from 0.67 to 0.77 for SSRs and 0.39 to 0.47 for

RAPDs (Thomas *et al.,* 1999). For both types of markers, over 94% of variation was found within populations. Planted stands did not differ significantly from naturally regenerated stands for expected heterozygosity.

The apparent discrepancy between selectively neutral genetic markers showing little population genetic differentiation, and polygenic morphological or physiological traits showing strong differentiation, is typical of widespread conifers. This is due in part to the homogenizing effects of long-distance gene flow *via* pollen and post-Pleistocene range expansions from glacial refugia on selectively neutral genetic markers, and in part to the strong effects of environment-dependent selection on adaptive traits (Cwynar and MacDonald, 1987; Delcourt and Delcourt, 1991). Yang *et al.* (1996) compared population differentiation for quantitative traits and allozymes for five populations of subsp. *latifolia*. They concluded that two branching traits which showed a low degree of population differentiation similar to allozyme (<6%), were likely selectively neutral (as allozymes are assumed to be), while size and wood specific gravity, with >13% of variation among populations, were under divergent selection.

Table 1. Summary of genetic diversity estimates for *Pinus contorta*

Populations Sampled [3]	Expected heterozygosity (H_e) within populations	% polymorphic loci	Population differentiation (F_{st} or G_{st})	Reference
Allozymes				
Rangewide (4 subsp.)			0.061 [1] 0.032 [2]	
Subsp. *latifolia*	0.118	69		Wheeler and Guries, 1982b
Subsp. *contorta*	0.126	65		
Subsp. *murrayana*	0.124	73		
Subsp. *bolanderi*	0.109	58		
Subsp. *latifolia*	0.194	69	0.034	Yang and Yeh, 1993
Subsp. *contorta*	0.180	62	0.057	
Subsp. *murrayana*	0.196	63	0.076	
Subsp. *contorta*, CA	0.119	38	0.057	Aitken and Libby, 1994
Subsp. *bolanderi*	0.105	28	0.044	
Subsp. *latifolia* – nearby populations CO	0.135	44	0.008	Knowles, 1984
Randomly Amplified Polymorphic DNA				
Subsp. *latifolia*, BC				
Central populations	0.160		0.081	Fazekas and Yeh, 2001
Intermediate populations	0.153		0.076	
Marginal populations	0.143		0.139	

[1] Location: USA - CA = California; CO = Colorado; Canada - BC = British Columbia
[2] Differentiation among populations within subspecies
[3] Differentiation among subspecies

Provenance testing and seedling genecological experiments are extensive for *Pinus contorta*, due to its widespread distribution, economic importance and use as an exotic. An enormous provenance trial established in British Columbia in the 1970s by the British Columbia Ministry of Forests included 142 populations and 60 field test sites (Xie and Ying, 1995; Rehfeldt *et al.,* 1999). A total of 158 seed lots were distributed around the world for International Union of Forest Research Organizations (IUFRO) provenance trials (summarized in K. Lindgren, 1993), but most of these trials contain only

a subset of provenances from a limited geographic area. The British Columbia provenance trial has been the focus of many published studies, and the trees under test are now over 20 years old. Population variation in growth rate, insect and disease resistance, shoot phenology, snow breakage and wood properties have been studied in these trials (Ying *et al.,* 1985; Ying and Hunt, 1987; Yanchuk *et al.,* 1988; O'Reilly and Owens, 1989; Xie and Ying, 1995; Wu *et al.,* 1996; Rehfeldt *et al.,* 1999). Most of the focus in this trial has been on subsp. *latifolia*, as survival and growth of the other subspecies is poor in the continental climate of interior British Columbia.

The results of the British Columbia provenance trial and seedling studies indicate that *P. contorta* is an adaptive specialist with locally adapted populations and relatively steep genetic clines, particularly associated with elevation of origin (Rehfeldt, 1987; Xie and Ying, 1995; Rehfeldt *et al.,* 1999). As the trees in the British Columbia field provenance trial aged, genetic clines became steeper over time with a greater proportion of the total variation due to differences among populations (Ying *et al.,* 1989; Xie and Ying, 1995). Seedling genecological studies have provided additional information on provenance variation in subsp. *latifolia* for adaptive traits including shoot growth components (phenology and rate of elongation), and cold hardiness (Rehfeldt, 1987, 1989; Lindgren and Nilsson, 1992; Chuine *et al.,* 2001). Genetic clines are strongly associated with source climate, with temperature rather than moisture variables accounting for the most population variation (Rehfeldt *et al.,* 1999). Populations originating from lower elevations have higher growth rates, longer growing seasons, greater resistance to needle cast and are more susceptible to snow breakage than those from higher elevations (Rehfeldt, 1987). Latitudinal clines are significant, but much weaker than those associated with elevation, with similar levels of differentiation observed for populations separated by 1000 m of elevation or 7° of latitude (Rehfeldt, 1987; Xie and Ying, 1995). Higher rates of height growth are related to a greater number of predetermined stem units, which result from a higher rate of initiation of primordia in buds during the previous growing season rather than a longer duration of initiation (Cannell and Willett, 1975; Chuine *et al.,* 2001).

Insect and disease resistance for a variety of pests including western gall rust (*Endocronartium harknessii)*, stalactiform blister rust (*Cronartium coleosporioides*), needle cast (*Lophodermella concolor)* and Sequoia pitch moth (*Synanthedon sequoiae*) varies significantly among provenances and increases with proximity to the natural range of *Pinus banksiana* (jack pine), indicating that introgression may provide genetic variation for pest defenses to *P. contorta* (Wu *et al.,* 1996). Susceptibility to all of these pests except sequoia pitch moth also increases with provenance elevation (Ying and Hunt, 1987; Yanchuk *et al.,* 1988; Wu *et al.,* 1996).

One test site in the British Columbia provenance trial was located on southern Vancouver Island in a location mild enough to allow for the survival and growth of all four subspecies. Even in this relatively mild location, the narrow adaptation of populations was observed, with all populations except those from Vancouver Island and nearby populations on the eastern tip of the Olympic Peninsula exhibiting poor growth or survival and declining vigour (Ying and Liang, 1994).

4.2.2. Variation among individuals within populations

Like most widespread conifers, there are high levels of within-population genetic variation in lodgepole pine for both genetic markers and quantitative traits. For allozyme loci, 91% of the total genetic variation resides within populations of lodgepole pine, while for many morphological and quantitative traits, a substantially greater proportion of variation is due to among-population variation (Wheeler and Guries, 1982b; Yang *et al.,* 1996; Table 1). Average expected heterozygosity estimates are typical of gymnosperms (Hamrick *et al.,* 1992). Expected heterozygosities within populations are similar for subsp. *latifolia*, subsp. *contorta* and subsp. *murrayana*, and lower for subsp. *bolanderi* (Wheeler and Guries, 1982b; Yang and Yeh, 1993; Aitken and Libby, 1994).

Nursery and field progeny trials for breeding programs of *P. contorta* subsp. *latifolia* have revealed significant variation among families within populations for height and diameter growth, branch length, angle and diameter, wood specific gravity, western gall rust infection and severity, stalactiform blister rust, needle cast, Sequoia pitch moth, ramicorn branch frequency, cold hardiness in North America, and weather injury and scleroderris canker (*Gremmeniella abietina*) infection in Sweden (Yanchuk *et al.*, 1988; Fries, 1989, 1991; Ericsson *et al.*, 1994; Ericsson and Danell, 1995; Wu *et al.*, 1995, 2000; Yang *et al.*, 1996, 1998; Ericsson and Andersson, 1997; Wu and Ying, 1997; Wang *et al.*, 1999, 2000). A sample of representative individual heritability estimates is provided in Table 2.

Early selection for growth and adaptive traits has been an area of interest to lodgepole pine breeders. Nursery-field and age-age correlations have been variable and often poor for growth (Wu *et al.,* 1997), cold hardiness (Ericsson and Andersson, 1997) and resistance to western gall rust (*Endocronartium harknessii*) (White *et al.,* 2000). However, combining early seedling nursery performance with field results may enhance selection efficiency and genetic gain (Wu *et al.,* 2000).

Table 2. Individual heritability estimates for growth and pest resistance traits in *Pinus contorta* subsp. contorta (lodgepole pine)

Trait	Location[1] and trial type	Individual heritability estimate or range	References
Height	Field progeny tests BC, AB, S	0.14-0.50	Rehfeldt, 1985; Ericsson and Danell, 1995; Xie and Ying, 1996; Yang *et al.*, 1998; Wu *et al.*, 2000
Height	Seedling greenhouse, BC	0.61	Wu *et al.*, 1995
Diameter	Field progeny test, BC	0.33-0.40	Xie and Ying, 1996; Wu *et al.*, 2000
Diameter	Seedling greenhouse, BC	0.46	Wu *et al.*, 1995
Harvest index	Field progeny test, BC	0.34	Wu *et al.*, 2000
Fall cold hardiness	Field progeny test, ID	0.10	Rehfeldt, 1989
Endocronartium harknessii infection	Field progeny test, BC and AB	0.12-0.50	Wu and Ying, 1997; Yang *et al.*, 1998
Cronartium coleosporioides infection	Field progeny test, BC	0.32	Wu and Ying, 1997
Lophodermella concolor impact	Field progeny test, BC	0.30	Wu and Ying, 1997
Synanthedon sequoiae damage	Field progeny test, BC	0.21	Wu and Ying, 1997
Injury/cankers, mainly *Gremmeniella abietina*	Field progeny test, S	0.12	Ericsson and Danell, 1995

[1] AB = Alberta, S = Sweden, ID =Idaho, BC = British Columbia

4.3. Inbreeding depression and genetic load

Self-fertility is low in lodgepole pine. Just 12% as many seeds on average were produced by controlled self-pollination as by controlled outcrossing, indicating very low self-fertility and high inbreeding depression for embryo survival, even for a conifer. This is likely a result of a high genetic load of recessive lethal alleles (Sorensen and Adams, 1993). In the same study, the relative self-fertility of a stand containing a small proportion of lodgepole pine in an ecologically marginal location for this species was almost twice that of more typical stands with higher proportions and densities of

this species, suggesting perhaps that mating system could shift to more self-pollination in a population with few founders through purging of recessive, deleterious alleles, *e.g.* following introduction as an exotic. The genetic load may be too high in particular cases for this to occur.

5. Hybridization

Pin*us contorta* subsp. *latifolia* hybridizes and introgresses with jack pine (*P. banksiana*) in both western Alberta and the Northwest Territories where the two closely related species are sympatric (Critchfield, 1980). The natural hybrid zone in northwestern Alberta has been studied for traits including cone orientation, curvature and prickles (Moss, 1949; Wagner *et al.*, 1991b), needle length (Keng and Little, 1961), chemical composition of turpentine (Mirov, 1956; Zavarin *et al.*, 1969) and a variety of allozyme and nuclear and organelle-based genetic markers (Wheeler and Guries, 1982b, 1987; Dancik and Yeh, 1983; Dong *et al.,* 1992; Wagner *et al.*, 1987, 1991b; Govindaraju *et al.*, 1988; Ye *et al.*, 2002). Despite the interspecific gene flow that introgression facilitates, the two species have remained quite distinct genetically in areas near the introgression zone. More widespread hybridization is prevented by phenological differences in female stroboli receptivity and pollen shed in these species, with lodgepole pine flowering 2 to 3 weeks later (Critchfield, 1985). The genetic distance between lodgepole pine and jack pine populations in Alberta based on allozymes averages 20 times greater than the genetic distance among populations within either species (Dancik and Yeh, 1983). Hybrids may have reduced reproductive rates relative to parental species: some artificial F1 hybrids of jack and lodgepole pine have high levels of pollen abortion, but F1 to F3 hybrids produce some sound seed (Critchfield, 1980). Each species does, however, show some influence of introgression from the other in natural populations near the introgression zone (Zavarin *et al.*, 1969). Some Alberta and Saskatchewan jack pine populations show lodgepole pine influence in morphology, chemistry, or mitochondrial DNA, but the degree of influence does not appear to be well correlated with distance from lodgepole pine (Critchfield, 1980; Dong and Wagner, 1993). Resistance of lodgepole pine to some insects and diseases is highest in the introgression zone and declines significantly with distance to the nearest populations of jack pine, suggesting that introgression may increase resistance in lodgepole pine (Wu *et al.,* 1996; Wu and Ying, 1998; Yang *et al.,* 1999). However, other explanations can be proposed for the clinal trends in lodgepole pine resistance. Yang *et al.* (1997) questioned whether the introgression interpretation is valid for western gall rust; neither study sampled non-hybrid jack pines. The extent to which introgression between these species occurred prior to, or since, the last glacial period is unclear (Critchfield, 1985; Dancik and Yeh, 1983).

Relatively strong reproductive barriers exist between both lodgepole pine and jack pine and the two other species in subsection *Contortae*, *P. virginiana* (Virginia pine) and *P. clausa* (sand pine), both native to the southeastern United States. Controlled crosses between lodgepole and Virginia pine have yielded only a few dwarfed, chlorotic hybrid progeny (Critchfield, 1985). Lodgepole pine has not been successfully hybridized with pines from any other subsections or continents. Repeated efforts have been made to hybridize *Pinus contorta* with *Pinus sylvestris* (subsection *Sylvestres*), but these have resulted in only empty or inviable seed (Duffield, 1951/1952; Critchfield, 1980). Attempts with other species in subsection *Sylvestres*, as well as western American hard pines in other subsections, have also failed (Duffield, 1951/1952; Critchfield, 1980). These strong barriers to hybridization prevent contamination of native pine gene pools in Europe, where *Pinus contorta* has been widely planted.

6. Ecology

6.1. Climate

Within its native range, lodgepole pine grows predominantly within boreal, temperate and mesothermal climates (Klinka *et al.,* 2000). Minimum temperatures range from 7°C on the coast at

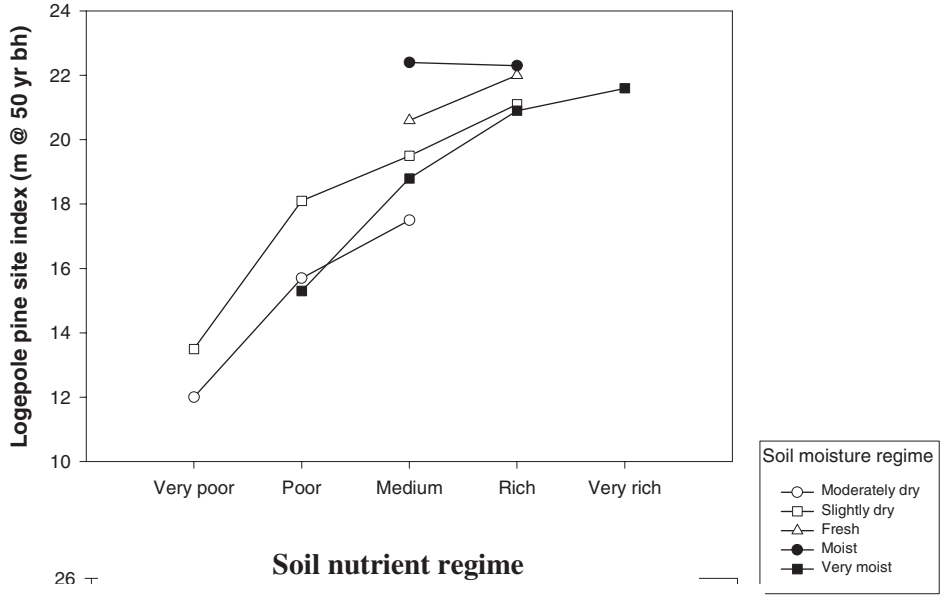
the southern edge of the species range to -57°C in the Northern Rocky Mountains (Lotan and Critchfield, 1990). Maximum temperatures range from 27°C for subsp. *contorta* along the coast and at high elevations to well over 38°C at low elevations for subsp. *latifolia* in the interior. Average July minimum temperatures are frequently below freezing at high elevations (Lotan and Critchfield, 1990). Lodgepole pine seedlings are cold hardy compared to many conifers, and can survive in low-lying 'frost-pockets' in some locations where other species do not (Cochran and Berntsen, 1973; Lotan and Perry, 1983). At low elevations in the interior, subsp. *latifolia* can grow in areas receiving as little as 250 mm of mean annual precipitation, whereas subsp. *contorta* receives more than 5,000 mm along the northern coast. Many subsp. *latifolia* and subsp. *murrayana* sites have low summer precipitation. Melting snow provides most of the soil water used by subsp. *latifolia* and *murrayana,* for rapid growth in early summer (Lotan and Critchfield, 1990).

6.2. Soils

Lodgepole pine grows across nearly the entire range of soil moisture conditions (from very dry to very wet) and soil nutrient conditions (from very poor to very rich), but the most productive growth occurs on fresh to moist, rich soils. Compared to many other tree species, it tolerates water-deficient, water-surplus, and nutrient-deficient soils well (Krajina, 1969; Klinka *et al.,* 2000).

Relationships between potential site index of lodgepole pine and analytical and categorical measures of site quality have been studied in the Sub-boreal Spruce (SBS) zone of British Columbia (Wang *et al.*, 1994; Klinka *et al.*, 1994, Kayahara *et al.*, 1995), and in the Boreal White and Black Spruce (BWBS) zone of the Upper Foothills natural subregion of Alberta (Brisco, 2001). Lodgepole pine site index (i) increased with increasing soil water supply from water-deficient to fresh and moist sites and then decreased with increasing water surplus, and (ii) increased from very poor through very rich sites with the rate of increase decreasing with increasing nitrogen availability. Increase in site index along the soil nutrient gradient was consistently steeper than along the soil moisture gradient (Figure 2).

Figure 2. Site index of lodgepole pine at 50 years (n = 235) in relation to soil nutrient regime (x axis) and soil moisture regime (symbols) across the Sub-boreal Spruce zone of British Columbia
(from Kayahara *et al.*, 1995)



Lodgepole pine site index appears to improve with increasing nitrogen availability even on water-deficient sites. All the trends in site index–site quality relationships are supported by regression analysis indicating that each soil moisture and nutrient regime had a strong relationship with site index. The best quantitative soil measure related to site index of lodgepole pine was forest floor and mineral soil C/N ratio, which explained about 40% in the variation of site index.

Brisco (2001) characterized soil nutrient regimes by several, predominantly nitrogen related measures (i.e., total N, mineralizable N, and forest floor C:N ratio and phosphorus, P) and determined foliar nutrient levels in young lodgepole pine stands across a range of sites. He found (i) significant differences in needle mass and foliar levels of N, P, and sulphur, S, between soil nutrient regimes, and (ii) a strong correlation between soil and foliar nutrient variables. In a relatively dry montane boreal climate (mean annual precipitation = 343 mm), soil moisture regime accounted for 63% and soil nutrient regime for 32% of the variation in lodgepole pine site index.

It can be concluded that both soil moisture and nitrogen are major determinants of tree growth in the SBS and BWBS zones, and that lodgepole pine will respond to nitrogen fertilization, with the response likely decreasing with increasing soil water surplus and available nitrogen. In general, lodgepole pine responds favourably to additions of nitrogen, and has shown a relatively consistent response to fertilization with various sources of nitrogen (Cochran, 1975; Cochran *et al.*, 1979; Yang, 1985; Brockley, 1989, 1990, 1996, 2001). On some sites nitrogen fertilization may induce sulphur or boron deficiencies.

Lodgepole pine grows well in many topographic positions. It grows well on both gentle slopes and in basins, but stands are also found on steep slopes, ridges, in rocky terrain and on a gravel substrate. The growth of lodgepole pine is better on northern and eastern slopes than on southern and western aspects (Alexander, 1974).

Lodgepole pine grows best where soil parent materials are derived from granites, shales, and coarse-grained lavas (Fowells, 1965). It is relatively rare on soils derived from limestone that tend to be dry; however, extensive stands occur on calcareous glacial tills (Smithers, 1961). Lodgepole pine grows well on glacial tills in Alberta, and it appears glacial drift provides a favourable balance of moisture and porosity. Highly calcareous soils derived from dolomitic limestone parent material in Montana usually do not support lodgepole pine (Lotan and Critchfield, 1990).

In the U.S. System of Soil Classification (U.S. Soil Conservation Service, 1975), extensive stands of *P. contorta* subsp. *latifolia* occur on Inceptisols and Alfisols in interior forests. Boralfs and Ochrepts probably support better tree development than Andepts, although lodgepole pine is common on the latter (Lotan and Critchfield, 1990). *Pinus contorta* subsp. *contorta*, is often found on Histosols (peat bogs or muskegs) in the hypermaritime forests of southeastern Alaska, coastal British Columbia, and western Washington, and on Inceptisols, Alfisols, and Ultisols on dry, sandy, or gravelly sites in more southern coastal areas (Lotan and Critchfield, 1990).

Lodgepole pine grows on wet flats and poorly drained soils, and can tolerate high water tables. These conditions often favour lodgepole pine over other tree species. In the Sierra Nevada, eastern Oregon, and coastal California, soils with an underlying hardpan support lodgepole pine and exclude species such as ponderosa pine (*Pinus ponderosa*), redwood (*Sequoia sempervirens*), or Douglas-fir (*Pseudotsuga menziesii*). On level sites in British Columbia, Alberta and central Oregon, the frost tolerance of lodgepole pine during germination allows its establishment but excludes other species (Lotan and Critchfield, 1990).

6.3. Synecology

Lodgepole pine grows predominantly in even-aged, post-fire forests in pure or, less often, mixed-species stands. It is a pioneer species on rock outcrops and in ombotrophic wetlands, and is present in early and mid-stages, and occasionally late stages, of secondary succession across a wide range of sites.

Owing to its wide climatic amplitude, lodgepole pine is a minor or major component in many regional ecosystems (climatic/vegetation zones); for example, in British Columbia, it occurs in all 12 forested biogeoclimatic zones (Krajina, 1969; Meidinger and Pojar, 1991). Given its wide edaphic amplitude, lodgepole pine is a minor or major but temporary component of many local ecosystems (plant associations, site types, habitat types, or forest cover types) (Krajina, 1969; Franklin and Dyrness, 1973; Eyre, 1980; Lotan and Critchfield, 1990; Meidinger and Pojar, 1991; Klinka *et al.*, 2000).

Lodgepole pine grows in extensive, pure stands delineated by the Lodgepole Pine forest cover type (Eyre, 1980), and is a component in 27 of the 55 western forest cover types. In montane boreal climates, it is represented in White Spruce (Type 201), White Spruce–Aspen (Type 251), White Spruce–Paper Birch (Type 202), Paper Birch (Type 252), and Black Spruce (Type 204) cover types. In subalpine boreal climates it is a component in all six high-elevation cover types: Mountain Hemlock (Type 205), Engelmann Spruce–Subalpine Fir (Type 206), Red Fir (Type 207), Whitebark Pine (Type 208), Bristlecone Pine (Type 209), and California Mixed Subalpine (Type 256). In cool temperate climates it is a minor component of seven other types: Interior Douglas-fir (Type 210), Western Larch (Type 212), Grand Fir (Type 213), Western White Pine (Type 215), Blue Spruce (Type 216), Aspen (Type 217), Limber Pine (Type 219), and Interior Ponderosa Pine (Type 237). In mesothermal climates it is a component in Coastal True Fir (Type 226), Western Redcedar–Western Hemlock (Type 227), Western Redcedar (Type 228), Douglas-fir–Western Hemlock (Type 230), Port Orford-Cedar (Type 231), Redwood (Type 232), and Jeffrey Pine (Type 247).

The cover and composition of understory vegetation in all these forest cover types varies and depends on site (climate and soil), associated tree species, stand developmental stage, and stand density. Relative to other tree species, light interception by lodgepole pine canopies is intermediate, thus providing light conditions for the development of diverse understory vegetation.

6.4. Stand dynamics

Lodgepole pine is intolerant of shade and competition from other tree species. Occasionally, seedlings establish and persist under a forest canopy and in small gaps, but these individuals rarely survive. In spite of its shade intolerance, lodgepole pine can survive in excessively dense stands for long periods, often for 50 years or more. Lodgepole pine typically regenerates after stand-destroying fires and develops even-aged, single-storied, single- or mixed-species stands. Four basic successional roles have been recognized by Pfister and Daubenmire (1975):

1. Minor seral: a component of even-aged stands rapidly being replaced by shade-tolerant associates in 50 to 200 years.
2. Dominant seral: the dominant component of even-aged stands with a vigorous understory of shade-tolerant species that will replace lodgepole pine in 100 to 200 years.
3. Persistent: the dominant component of even-aged stands with little evidence of replacement by shade-tolerant species.
4. Climax: the only tree species capable of growing in a particular environment; lodgepole pine is self-perpetuating (e.g., in the Sub-boreal Pine – Spruce zone of British Columbia (Meidinger and Pojar, 1991) and in the *Pinus contorta* zone of Oregon (Franklin and Dyrness, 1973).

In the absence of fire, lodgepole pine is usually succeeded by its more tolerant associates, such as white spruce (Picea glauca), Engelmann spruce (Picea engelmannii), and subalpine fir (Abies lasiocarpa). Succession proceeds at variable rates, and is particularly slow in some high elevation forests. Pure stands of lodgepole pine persist for varying lengths of time. Low-elevation stands begin to break up at 80 to 100 years, while high-elevation stands last for several hundred years. For example, pure stands in and around Yellowstone National Park contain 300 to 400-year-old trees, with several groups of younger even-aged trees. These stands originated as even-aged stands but have been breaking up for centuries (Lotan and Critchfield, 1990). A typical lifespan of some lodgepole pines can be 250 to 600 years.

The ability of lodgepole pine to regenerate at the expense of other species is due to cone serotiny, seed viability, germinative energy, rapid juvenile growth, and ability to survive a wide variety of climate, microsite and soil conditions (Lotan, 1976). Lodgepole pine responds positively to thinning at an early age (Cole, 1975). Heavily stocked managed stands must be thinned to prevent stagnation. Overstocked stands on poor sites should be thinned as early as age 10.

6.5. Damaging agents

Lodgepole pine is host to a large number of insects and diseases. The mountain pine beetle (*Dendroctonus ponderosae*) is the most severe insect pest of lodgepole pine. The epidemics that periodically occur in many lodgepole pine stands seriously impact long-term yield. Adult beetles attack trees in July or August, introducing blue stain fungi (Amman, 1978). The mature insects form egg galleries in the phloem, and larvae feed in these galleries. The beetles and fungi together girdle and kill trees. Larvae over-winter in the tree, complete development, and emerge as adult beetles in the spring. Harvesting has been considered a means of preventing mountain pine beetle epidemics (Cole, 1978), and no mortality occurred in heavily thinned stands in Oregon where vigour ratings were high (Mitchell *et al.,* 1981). However, mountain pine beetle has killed lodgepole pine across a wide range of stand ages and densities in the epidemic that started in the late 1990s in British Columbia. The mountain pine beetle has played an historic role in the dynamics of lodgepole pine ecosystems. Through periodic epidemics, large amounts of fuel are produced, which eventually burn, generating favourable conditions for lodgepole pine regeneration (Brown, 1975; Lotan, 1976).

There are a number of other insects that can damage lodgepole pine locally. The lodgepole terminal weevil (*Pissodes terminalis*) destroys elongating terminal leaders. Larvae of the Warren's collar weevil (*Hylobius warreni*) girdle roots and the root collar. Larvae of the weevil *Magdalis gentilis* mine and kill branches. Lodgepole pine is host to a number of sucking insects, including the pine needle scale (*Chionaspis pinifoliae*), the black pineleaf scale (*Nuculaspis californica*), and the spruce spider mite (*Oligonychus ununguis*). Several insects defoliate lodgepole pine, including lodgepole sawfly (*Neodiprion burkei*), the lodgepole needle miner (*Coleotechnites milleri*), the sugar pine tortrix (*Choristoneura lambertiana*), the pine tube moth (Argyrotaenia pinatubana), and the pandora moth (*Coloradia pandora*) (Amman, 1975; Lotan and Critchfield, 1990). In plantations in central Europe, pine shoot moths (*Rhyacionia buoliana* and other related species) are important insect pest (Stephan, 1980).

Lodgepole pine is seriously affected by the parasite dwarf mistletoe (particularly *Arceuthobium americanum*) (Baranayay, 1975; Hawksworth, 1975). Sticky dwarf mistletoe seeds are forcibly ejected as far as 9 m, and adhere to the foliage of neighbouring trees. The proportion of trees infected can increase rapidly over time (Hawksworth, 1975). Dwarf mistletoe can spread in young stands at 0.3 to 0.5 m per year, with the fastest spread in dense stands. In many areas, over 50% of lodgepole pine forests are infected (Lotan and Critchfield, 1990). Infections reduce growth and vigour, increase mortality, reduce wood quality, and decrease seed production. Dwarf mistletoe can be managed by clearcutting units designed to reduce infection of regeneration from surrounding stands. Fire can also limit spread of dwarf mistletoe by eliminating sources of infection. (Lotan and Critchfield, 1990).

Lodgepole pine is subject to attack by many fungal pathogens that can reduce growth and cause mortality (Krebill, 1975). *Atropellis piniphila* causes a stem canker that is one of the most serious diseases in lodgepole pine, and renders stems useless for most solid wood uses. Rust fungi causing stem cankers result in mortality, reductions in growth, and log defects. Comandra blister rust (*Cronartium comandrae*) is the most serious of these. Western gall rust, caused by the fungus *Peridermium harknessii*, causes trunk cankers that result in log culls and seedling and sapling mortality. This rust does not have an alternate host, thus can directly re-infect pines. Needle casts are caused by fungi including *Elytroderma deformans* and *Lophodermella concolor*. Lodgepole pine is susceptible to root rots caused by fungi including *Armillaria ostoyae* and *Heterobasidion annosum*. Wood decay results from fungi such as *Phellinus pini* and *Peniophora pseudo-pini*. The fungus *Gremmeniella abietina* is an important damaging agent of lodgepole pine in Europe, causing stem cankers (Karlman, 1993; Witzell and Karlman, 2000).

Warm, dry Chinook winds following extremely cold weather occasionally cause winter desiccation, known as red belt injury, particularly in Canada and Montana. The resulting defoliation of trees is common, and mortality can occur over large areas. Heavy snow can break or bend trees, particularly in dense stands. Thinning of dense stands can increase snow breakage (Lotan and Critchfield, 1990).

7. Forestry practices

7.1. Deployment of reforestation materials

Lodgepole pine is one of the most commonly regenerated trees in Western North America. The area planted, primarily using containerized seedling stock, surpasses that regenerated naturally. It can be grown in single- or mixed-species stands, preferably with shade-tolerant species. Depending on site and management objectives, clearcutting and patch-cutting systems are viable silvicultural systems for the establishment and growth of the species (Burns, 1983). Propagation by seed is currently the primary method for regenerating lodgepole pine. Techniques for collection, processing, testing, and storage of seed are given in Schopmeyer (1974). The annual planting of lodgepole pine as of 1992 was 70 million seedlings in British Columbia, 8 million in Alberta, 3 million in Idaho and Montana combined, and small programs in the Pacific Northwestern United States and Alaska (D. Lindgren, 1993). Planting in British Columbia has increased since that time.

Lodgepole pine was first introduced to Europe in 1832. It was planted in arboreta, parks, and on a minor scale in forests. In 1950, lodgepole pine became a major species for afforestation of peatlands in Britain, Ireland, Sweden, and Finland. Today, lodgepole pine plantations also exist in the Netherlands, Denmark, Island, Norway, Germany, Poland, and the former Soviet Union. The country with the largest share of exotic plantations of lodgepole pine is Sweden due to the superior growth and cold hardiness of this species compared to *Pinus sylvestris* (K. Lindgren, 1993). Interest in lodgepole pine as an exotic has declined in recent years for a variety of reasons, including naturalization and invasiveness in New Zealand (Ledgard, 1993), insect problems in the Netherlands (de Vries, 1993), and changes in policy regarding exotic species and risks in Sweden (Lindgren *et al.,* 1993).

7.2. Provenance transfer

Lodgepole pine is considered an adaptational specialist rather than generalist, meaning that populations differ genetically over fairly short physical or environmental distances (Rehfeldt, 1988; Xie and Ying, 1995). Thus, the seed transfer limits for this species are relatively conservative. In British Columbia, natural stand seed of *Pinus contorta* subsp. *latifolia* can be moved 2° latitude to the north, 1° south, 3° longitude to the west, 2° east, 300 m up in elevation and 100 m down in elevation from the location of collection to the planting site. Seed transfer guidelines are asymmetrical since results from provenance trials indicate that sources from slightly milder locations (lower elevation or farther south)

show slightly superior growth rates with no increase in mortality compared to local provenances. If seed is transferred between seed planning zones, it must be moved to the same ecosystem type it was collected from. Six superior provenances have been identified in British Columbia based on provenance trial results, and allowable seed transfer distances are greater for these than for other provenances (BC Ministry of Forests, 1995; Xie and Ying, 1995).

In Alberta, natural stand seed collected for reforestation from all forest tree species including lodgepole pine must be used within 80 km and 150 m elevation of the collection site. In Sweden, forest tree seed is managed within six seed zones, defined by latitude and elevation, and seed orchards are designated for each zone (Ericsson, 1993). Parent trees of seedlings in seedling seed orchards or grafted clones in clonal orchards for different Swedish zones originate from different geographic areas in British Columbia and Yukon Territory.

7.3. Breeding programmes

There are active breeding programs for lodgepole pine (*P. contorta* subsp. *latifolia*) in British Columbia, Alberta, the Inland Empire of the United States and northern Europe. The largest program is in British Columbia, with six active breeding programs for different geographic areas (breeding zones), and 14 seed orchards serving these areas (Forest Genetics Council of B.C, 2001). There are 100 to 500 parent trees undergoing progeny testing in each of these zones. The primary trait for improvement is stem volume, accomplished through indirect selection on tree height due to the much higher heritability of height than diameter or volume, while maintaining density is a secondary goal (Wang *et al.,* 1999). Current gains for volume range from 6 to 11% (Forest Genetics Council of B.C, 2001). Most seed orchards are located in the Okanagan Valley, a warm, arid region, and early seed production was lower than expected due to a climate-related pollination or fertilization problem.

In Alberta, there are five breeding zones with a total of approximately 1,100 phenotypically selected parent trees, and most of these have included progeny tests (Dhir and Barnhardt, 1993). Two additional breeding zones are under development (N. Dhir, Alberta Forest Service, pers. comm.). A combination of seedling and grafted clonal seed orchards have been established to produce improved seed from selected parent trees. In addition to survival, growth and wood density, susceptibility to western gall rust (*Endocronartium harknessii*) is being evaluated (Yang *et al.,* 1997).

In Idaho and Montana, the Inland Empire Tree Improvement Cooperative has had an ongoing breeding program since 1978. Approximately 1,000 phenotypically-selected parent trees are represented in open-pollinated progeny tests. A series of short-term nursery trials of additional phenotypic selections were established in 1992 (D. Lindgren, 1993).

In Sweden, the breeding program is based on approximately 1,100 open-pollinated families of subsp. *latifolia* established in both seedling seed orchards and progeny trials (Ericsson *et al.,* 1994; Ericsson and Danell, 1995). Seedling and grafted clonal seed orchards were established, with 140 ha of seed orchards as of 1992. Seed production from these orchards exceeds current planting requirements in Sweden to the point where seed could be exported (Lindgren *et al.,* 1993). Field tests are evaluated for health, height, and ramicorn branch frequency at 8 to 12 years of age, while damage caused by the fungus *Gremmeniella abietina* and weather-related injuries are evaluated on harsh sites (Ericsson and Danell, 1995). Roguing orchards based on the results should produce genetic gains of 2 to 6%.

D. Lindgren (1993) summarized the status of breeding programs of lodgepole pine around the world. At that time there were small breeding programs in Norway (subsp. *latifolia*), Ireland (subsp. *contorta*), Britain (subsp. *contorta*) and Finland (subsp. *latifolia*). There was a substantial program underway in the Pacific Northwestern United States (subsp. *latifolia*), but with a shift away from even-aged management and artificial regeneration, this program has been reduced. There are no known breeding programs for subsp. *murrayana* or subsp. *bolanderi*.

7.4. Conservation of genetic resources

Lodgepole pine is a widespread species with high fecundity and high population densities. Natural regeneration is relied upon extensively in some portions of its range. It is well-represented in parks and ecological reserves throughout its native range. Thus, threats to genetic diversity are low for this species. Lester and Yanchuk (1996) concluded that subsp. *latifolia* and subsp. *contorta* were both well-protected in existing protected areas in British Columbia, with the exception of the Tatshenshini Basin, Alberta Plateau and Fort Nelson Lowland in northern BC; protected areas in the province have nearly doubled since that assessment. Sierra Nevada lodgepole pine, subsp. *murrayana*, is in high-elevation areas of the Sierra Nevada Mountains of California, an area that is well-represented in National Parks and US Forest Service Wilderness Areas, and otherwise undergoes little harvesting. Bolander pine, subsp. *bolanderi*, endemic to the Mendocino pygmy forest, is not harvested for wood but is under some pressure due to residential development. There is a substantial ecological reserve as well as a California state park and a privately owned conservation reserve in this small area for *in situ* gene conservation. Shore pine, var. *contorta*, is represented in coastal parks and ecological reserves from California to Alaska, and is not harvested commercially over much of its range. Additionally, the extensive provenance trials, other genetic tests, breeding arboreta and seed banks provide *ex situ* gene conservation, particularly for subsp. *latifolia*. Climate change may result in maladaptation of populations in reserves, but Rehfeldt *et al.* (1999) predicted that lodgepole pine populations would adapt to predicted levels of climate change in 4 to 12 generations as a result of natural selection.

8. Summary

Lodgepole pine has one of the widest ecological amplitudes of any conifer in North America and is one of the important and valuable timber crop species in Western North America and northern Europe. Across most of its range, it is a pioneer and early seral, short-lived, and fire-adapted species. Because of the rapid growth rate, low taper, thin bark, and relatively narrow crown, it produces a higher volume of wood sooner than many of its associates. It has low nutrient requirements and is easy to regenerate and grow. A common problem of regenerating lodgepole pine is overstocking, which may result in growth stagnation in early stand development on water-deficient, nutrient-poor sites.

The ecology of lodgepole pine is diverse as a result of its large geographic distribution. Although it typically occurs in pure, even-aged stands, lodgepole pine associates with a great number of shade-tolerant tree species, especially in late seral stages, and is a minor or major component in many ecosystems in many climatic zones in Western North America. Lodgepole pine is not only an important timber species, but is also a major tree species in many scenic and recreational areas, and on critical watersheds. It provides a large area of wildlife habitat and is widely associated with grazing and range allotments.

Lodgepole pine is an adaptive specialist, with populations varying with climatic gradients in temperature, and to a lesser extent, moisture. Appropriate seed transfer distances are short for all except a few more broadly adapted provenances. Breeding programs must start with well-adapted, local populations in order to obtain genetic gain. Genetic variation within populations is high, offering opportunities for obtaining genetic gain in growth and wood quality traits. While variation for disease and insect resistance is high, the use of locally adapted populations and planting of this species on appropriate sites will adequately manage pest problems from a genetic standpoint in most cases. The genetic resources of this species are generally well-protected by *in situ* reserves throughout its natural range, although adaptation of these populations to a rapid change in climate may take several to many generations.

Lodgepole pine has received relatively little study in terms of genetic transformation. While methods for genetic engineering and regeneration of transgenic plants through somatic embryogenesis developed for short-rotation pines could likely be adapted for this species, the long rotation lengths and use of locally-adapted populations, as well as a lack of single-gene traits of interest, make the use of this technology unlikely for operational reforestation in the near future.

References

Aitken, S.N. and W.J. Libby. 1994. Evolution of the pygmy-forest edaphic subspecies of *Pinus contorta* across an ecological staircase. Evolution 48: 1009-1019.

Alexander, R.R. 1974. Silviculture of subalpine forest in the central and southern Rocky Mountains: The status of our knowledge. USDA Forest Service Research Paper RM-121, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado. 88 pp.

Amman, G.D. 1975. Insects affecting lodgepole pine productivity. Pp. 310-341 *in* Proceedings, Symposium on Management of Lodgepole Pine Ecosystems, October 9-11, 1973. Washington State University, Pullman.

Amman, G.D. 1978. Biology, ecology, and causes of outbreaks of the mountain pine beetle in lodgepole pine forests. Pp. 39-53 *in* Proceedings of Symposium on Theory and Practice of Mountain Pine Beetle Management in Lodgepole Pine Forests, Washington State University, Pullman, Washington.

Baranyay, J.A. 1975. Dwarf mistletoe as a factor in the management of lodgepole pine forests in western Canada. Pp. 359-376 *in* Proceedings of Symposium on Theory and Practice of Mountain Pine Beetle Management in Lodgepole Pine Forests, Washington State University, Pullman, Washington.

Brisco, D.J. 2001. Relationships between stand and site factors in naturally established fire-origin lodgepole pine stands in the Upper Foothills of Alberta. M.Sc. thesis, University of British Columbia, Vancouver, B.C. 83 pp.

British Columbia Ministry of Forests. 1995. Seed and vegetative material guidebook. Forest Practices Code of British Columbia. Victoria, B.C. 57 pp.

Brockley, R.P. 1989. Response of thinned, immature lodgepole to nitrogen fertilization: Three-year growth response. FRDA Report 036, British Columbia Ministry of Forests, Victoria, B.C. 37 pp.

Brockley, R.P. 1990. Response of thinned immature lodgepole pine to nitrogen and boron fertilization. Canadian Journal of Forest Research 20: 579-585.

Brockley, R.P. 1996. Lodgepole nutrition and fertilization: A summary of British Columbia Ministry of Forests research results. FRDA Report 266, British Columbia Ministry of Forests, Victoria, B.C. 43 pp.

Brockley, R.P. 2001. Foliar sampling guidelines and nutrient interpretive criteria for lodgepole pine. Extension Note, British Columbia Ministry of Forests, Victoria, B.C. 8 pp.

Brown, J.K. 1975. Fire cycles and community dynamics in lodgepole pine forests. Pp. 429-456 *in* Proceedings of Symposium on Theory and Practice of Mountain Pine Beetle Management in Lodgepole Pine Forests, Washington State University, Pullman, Washington.

Burdon, R.D. 2002. An introduction to pines. Pp. x-xxi *in* CABI Forestry Compendium (ed.), Pines of silvicultural importance. CABI Publishing. Wallingford, U.K.

Burns, R.M. (technical compiler). 1983. Silvicultural systems for the major forest types of the United States. USDA Agriculture Handbook No. 445, Washington, D.C. 191 pp.

Cannell, M.G.R. and S.C. Willett. 1975. Rates and times at which needles are initiated in buds on different provenances of *Pinus contorta* and *Picea sitchensis*. Canadian Journal of Forest Research 5: 367-380.

Chuine, I., S.N. Aitken and C. Ying. 2001. Temperature-dependent growth in provenances of *Pinus contorta*. Canadian Journal of Forest Research 31: 1444-1455.

Cochran, P.H. 1975. Response of pole-size lodgepole pine to fertilization. USDA Forest Service Research Note PNW-247, Pacific Northwest Forest and Range Experiment Station, Portland, Oregon. 10 pp.

Cochran, P.H. and C.M. Berntsen. 1973. Tolerance of lodgepole pine and ponderosa pine seedlings to low night temperatures. Forest Science 19: 272-280.

Cochran, P.H., W. Lopushinsky, E.C. Steinbrenner and S.R. Webster. 1979. Response of ponderosa pine and lodgepole pine to fertilization. Pp. 89-94 *in* S.P. Gessel, R.M. Kenady and W.A. Atkinson (eds.), Proceedings,

Forest Fertilization Conference, Contribution No. 40, College of Forest Resources, University of Washington, Seattle, Washington.

Cole, D.M. 1975. Culture of immature lodgepole stands for timber objectives. Pp. 536-555 *in* Proceedings of Symposium on Management of Lodgepole Pine Ecosystems. Washington State University, Pullman, Washington.

Cole, D.M. 1978. Feasibility of silvicultural practices for reducing losses to the mountain pine beetle in lodgepole pine forests. Pp. 140-147 *in* Proceedings of Symposium on Theory and Practice of Mountain Pine Beetle Management in Lodgepole Pine Forests, Washington State University, Pullman, Washington.

Critchfield, W.G. 1957. Geographic variation in *Pinus contorta*. Maria Moors Cabot Foundation Publication No. 3. Harvard University, Cambridge, Massachusetts. 118 pp.

Critchfield, W.B. 1980. Genetics of lodgepole pine. USDA Forest Service, Research Paper WO-37, Washington, D.C. 57 pp.

Critchfield, W.B. 1984. Impact of the Pleistocene on the genetic structure of North American conifers. Pp. 70-114 *in* R.M. Lanner (ed.), Proceedings of the 8th North American Forest Biology Workshop. Department of Forest Resources, Utah State University, Logan, Utah.

Critchfield, W.B. 1985. The late Quaternary history of lodgepole and jack pines. Canadian Journal of Forest Research 15: 749-772.

Cwynar, L.C. and G.M. MacDonald. 1987. Geographical variation of lodgepole pine in relation to population history. American Naturalist 129: 463-469.

Dahms, W.G. 1963. Dispersal of lodgepole pine seed into clearcut patches. USDA Forest Service Research Note PNW-3, Pacific Northwest Forest and Range Experiment Station, Portland, Oregon. 7 pp.

Dahms, W.G. and J.W. Barrett. 1975. Seed production of central Oregon ponderosa and lodgepole pines. USDA Forest Service Research Paper PNW-19, Pacific Northwest Forest and Range Experiment Station, Portland, Oregon. 13 pp.

Dancik, B.P. and F.C. Yeh. 1983. Allozyme variability and evolution of lodgepole pine (*Pinus contorta* var. *latifolia*) and jack pine (*Pinus banksiana*) in Alberta. Canadian Journal of Genetics and Cytology 25: 57-64.

Delcourt, H.R. and P.A. Delcourt. 1991. Quaternary ecology: A paleoecological perspective. Chapman & Hall, London, U.K.

De Vries, S.M.G. 1993. IUFRO provenances of *Pinus contorta* in The Netherlands. *In* D. Lindgren (ed.), Proceedings of Pinus contorta – From untamed forest to domesticated crop. Meeting of IUFRO WP 2.02.06 and Frans Kempe Symposium, Umeå, August 24-28, 1992. Swedish University of Agricultural Sciences, Report 11: 209-210.

Dhir, N.K. and L.K. Barnhardt. 1993. Genetic improvement of lodgepole pine in Alberta. *In* D.Lindgren (ed.), Proceedings of Pinus contorta – From untamed forest to domesticated crop. Meeting of IUFRO WP 2.02.06 and Frans Kempe Symposium, Umeå, August 24-28, 1992. Swedish University of Agricultural Sciences, Report 11: 279-288.

Dong, J. and D.B. Wagner. 1993. Taxonomic and population differentiation of mitochondrial diversity in *Pinus banksiana* and *Pinus contorta*. Theoretical and Applied Genetics 86: 573-578.

Dong, J. and D.B. Wagner. 1994. Paternally inherited chloroplast polymorphism in *Pinus*: Estimation of diversity and population subdivision, and tests of disequilibrium with a maternally inherited mitochondrial population. Genetics 136: 1187-1194.

Dong, J., D.B. Wagner, A.D. Yanchuk, M.R. Carlson, S. Magnussen, X.-R. Wang and A.E. Szmidt. 1992. Paternal chloroplast DNA inheritance in *Pinus contorta* and *Pinus banksiana*: independence of parental species or cross direction. Journal of Heredity 83: 419-422.

Duffield, J.W. 1951/1952. Relationships and species hybridization in the genus *Pinus*. Zeitschrift für Forstgenetik und Forstpflanzenzüchtung 1: 93-100.

Epperson, B.K. and R.W. Allard. 1984. Allozyme analysis of the mating system in lodgepole pine. Journal of Heredity 75: 212-214.

Ericsson, T. 1993. Provenance qualities of the *Pinus contorta* breeding base in Sweden. SkogForsk (The Foresty Research Institute of Sweden) Report No. 4. 33 pp.

Ericsson, T. and O. Danell. 1995. Genetic evaluation, multiple-trait selection criteria and genetic thinning of *Pinus contorta* var. *latifolia* seed orchards in Sweden. Scandinavian Journal of Forest Research 10: 313-325.

Ericsson, T. and B. Andersson. 1997. Can field mortality of lodgepole pine (*Pinus contorta* var. *latifolia*) families be predicted based on early freezing test results? Forest Genetics 4: 61-67.

Ericsson, T., O. Danell and B. Andersson. 1994. Genetic variation of *Pinus contorta* var. *latifolia* breeding material in Sweden. Canadian Journal of Forest Research 24: 723-729.

Eyre, F.H. (ed.) 1980. Forest types of the United States and Canada. Society of American Foresters, Washington, DC. 148 pp.

Fazekas, A.J. and F.C. Yeh. 2001. Random amplified polymorphic DNA diversity of marginal and central populations in *Pinus contorta* ssp. *latifolia*. Genome 44: 13-22.

Forest Genetics Council of British Columbia. 2001. Business Plan 2001/2002. Forest Genetics Council of British Columbia, Victoria, B.C. 19 pp. plus appendices.

Fowells, H.A. (compiler) 1965. Silvics of forest trees of the United States. USDA Agriculture Handbook No. 271, Washington, D.C. 762 pp.

Franklin, J.F. and C.T. Dyrness. 1973. Natural vegetation of Oregon and Washington. USDA Forest Service General Technical Report PNW-8, Pacific Northwest Forest and Range Experiment Station, Portland, Oregon. 416 pp.

Fries, A. 1989. Genetic parameters of 2-year-old full-sib families of Pinus contorta and correlation with field test data. Scandinavian Journal of Forest Research 4: 137-147.

Fries, A. 1991. Nursery growth and dry weight of plus tree progenies of *Pinus contorta*. Scandinavian Journal of Forest Research 6: 161-176.

Fries, A. and Z. Kaya. 1997a. Genetic control of rooting ability of lodgepole pine cuttings. Forest Science 43: 582-588.

Fries, A. and Z. Kaya. 1997b. Parameters affecting shoot production and rooting of cuttings from lodgepole pine hedges. New Forests 12: 101-111.

Govindaraju, D.R., D.B. Wagner, G.P. Smith and B.P. Dancik. 1988. Chloroplast DNA variation within individual trees of a *Pinus banksiana* – *Pinus contorta* sympatric region. Canadian Journal of Forest Research 18: 1347-1350.

Griffin, J.R. and W.B. Critchfield. 1976. The distribution of forest trees in California. USDA Forest Service Research Paper PSW-82 (reprint with supplement), Pacific Southwest Forest and Range Experiment Station, Berkeley, California. 118 pp.

Hamrick, J.L., M.J.W. Godt and S.L. Sherman-Boyles. 1992. Factors influencing levels of genetic diversity in woody plant species. New Forests 6: 95-124.

Hawksworth, F.G. 1975. Dwarf mistletoe and its role in lodgepole pine ecosystems. Pp. 342-358 *in* Proceedings, Symposium on Theory and Practice of Mountain Pine Beetle Management in Lodgepole Pine Forests, Washington State University, Pullman, Washington.

Hicks, M., D. Adams, S. O'Keefe, E. Macdonald and R. Hodgetts. 1998. The development of RAPD and microsatellite markers in lodgepole pine (*Pinus contorta* var. *latifolia*). Genome 41: 797-805.

Karlman, M. 1993. The *Gremmeniella* disease situation on lodgepole pine in northern Sweden. *In* D.Lindgren (ed.), Proceedings of Pinus contorta – From untamed forest to domesticated crop. Meeting of IUFRO WP 2.02.06 and

Frans Kempe Symposium, Umeå, August 24-28, 1992. Swedish University of Agricultural Sciences, Report 11: 335-349.

Kayahara, G.J., K. Klinka and I. Moss. 1995. Site index–site quality relationships for lodgepole pine, interior spruce, and subalpine fir in the Sub-boreal Spruce zone of British Columbia. Contract Report to Northwood Pulp and Timber Ltd., Prince George, B.C. 24 pp.

Keng, H. and E.L. Little Jr. 1961. Needle characteristics of hybrid pines. Silvae Genetica 10: 131-146.

Klinka, K., Q. Wang and G.J. Kayahara. 1994. Quantitative characterization of nutrient regimes in some boreal forest soils. Canadian Journal of Soil Science 74: 29-38.

Klinka K., J. Worrall, L. Skoda and P. Varga. 2000. The distribution and synopsis of ecological and silvical characteristics of tree species of British Columbia's forests. Can. Cartographics Ltd., Coquitlam, B.C. 180 pp.

Knowles, P. 1984. Genetic variability among and within closely spaced populations of lodgepole pine. Canadian Journal of Genetics and Cytology 26: 177-184.

Krajina, V.J. 1969. Ecology of forest trees in British Columbia. Ecol. West. North Am. 2: 1-146.

Kral, R. 1993. *Pinus* Linnaeus. Pp. 373-398 *in* Flora of North America Editorial Committee (ed.), Flora of North America North of Mexico, Vol. 2: Pteridophytes and Gymnosperms. Oxford University Press, New York.

Krebill, R.G. 1975. Lodgepole pine's fungus-caused diseases and decays. Pp. 377-405 *in* Proceedings of Symposium on Management of Lodgepole Pine Ecosystems, Washington State University, Pullman, Washington.

Ledgard, N.J. 1993. A review of research and management of natural regeneration of introduced lodgepole pine (*Pinus contorta* Dougl.) with particular emphasis on its unwanted spread in New Zealand. *In* D. Lindgren, (ed.), Proceedings of *Pinus contorta* – From untamed forest to domesticated crop. Meeting of IUFRO WP 2.02.06 and Frans Kempe Symposium, Umeå, August 24-28, 1992. Swedish University of Agricultural Sciences, Report 11: 388-406.

Lester, D.T. and A.D. Yanchuk. 1996. A survey of the protected status of conifers in British Columbia: *In situ* gene conservation. Province of British Columbia, Ministry of Forests Research, Program Research Report 04. 34 pp.

Lindgren, D. 1993. Breeding *Pinus contorta* in different countries. *In* D. Lindgren (ed.), Proceedings of *Pinus contorta* – From untamed forest to domesticated crop. Meeting of IUFRO WP 2.02.06 and Frans Kempe Symposium, Umeå, August 24-28, 1992. Swedish University of Agricultural Sciences, Report 11: 264-270.

Lindgren, D., K. Lindgren and P. Krutzsch. 1993. Use of lodgepole pine and its provenances in Sweden. *In* D. Lindgren (ed.), Proceedings of *Pinus contorta* – From untamed forest to domesticated crop. Meeting of IUFRO WP 2.02.06 and Frans Kempe Symposium, Umeå, August 24-28, 1992, Swedish University of Agricultural Science, Report 11: 238-263.

Lindgren, K. 1993. IUFRO *Pinus contorta* seed collections: Distribution and publications. In D. Lindgren (ed.), *Proceedings of Pinus contorta* – From untamed forest to domesticated crop. Meeting of IUFRO WP 2.02.06 and Frans Kempe Symposium, Umeå, August 24-28, 1992. Swedish University of Agricultural Sciences, Report 11: 232-237.

Lindgren, K. and J.-E. Nilsson. 1992. Cold acclimation of *Pinus sylvestris* and *Pinus contorta* provenances as measured by freezing tolerance of detached needles. Scandinavian Journal of Forest Research 7: 309-315.

Little Jr., E.L. 1979. Checklist of United States trees (native and naturalized). USDA Agriculture Handbook No. 541, Washington, D.C. 375 pp.

Little Jr., E.L. and W.B. Critchfield. 1969. Subdivisions of the genus *Pinus* (pines). USDA Forest Service Miscellaneous Publication No. 1144. 51 pp.

Lotan, J.E. 1964. Initial germination and survival of lodgepole pine on prepared seedbeds. USDA Forest Service Research Note INT-29, Intermountain Forest and Range Experiment Station, Ogden, Utah. 8 pp.

Lotan, J.E. 1975. The role of cone serotiny in lodgepole pine forests. Pp. 471-495 *in* Proceedings of Symposium on Management of Lodgepole Pine Ecosystems. Washington State University, Pullman, Washington.

Lotan, J.E. 1976. Cone serotiny - fire relationships in lodgepole pine. Pp. 267-278 *in* Proceedings, 14th Annual Tall Timbers Fire Ecology Conference, Tall Timbers Research Station, Tallahassee, Florida.

Lotan, J.E. and D.A. Perry. 1983. Ecology and regeneration of lodgepole pine. USDA Agriculture Handbook No. 606, Washington, D.C. 51 pp.

Lotan, J.E. and W.B. Critchfield. 1990. *Pinus contorta* Dougl. *ex* Loud. Pp. 302-315 i*n* R.M. Burns and B.H. Honkala (technical coordinators). Silvics of North America, Vol. 1. USDA Agriculture Handbook No. 654, Washington, D.C.

MacDonald, G.M. and L.C. Cwynar. 1985. A fossil pollen based reconstruction of the late Quaternary history of lodgepole pine (*Pinus contorta* ssp. *latifolia*) in the western interior of Canada. Canadian Journal of Forest Research 15: 1039-1044.

Meidinger, D.V. and J. Pojar (compilers and eds.). 1991. Ecosystems of British Columbia. Special Report Series 6, British Columbia Ministry of Forests, Victoria, B.C. 330 pp.

Mitchell, R.G., R.H. Waring and G.B. Pitman. 1981. Thinning lodgepole in Oregon to increase tree vigour and reduce mountain pine beetle damage. Unpublished report, Oregon State University, Corvallis, Oregon.

Mirov, N.T. 1956. Composition of turpentine of lodgepole x jack pine hybrids. Canadian Journal of Botany 34: 443-457.

Mirov, N.T. and J. Hasbrouck. 1976. The story of pines. Indiana University Press, Bloomington, Indiana. 148 pp.

Moss, E.H. 1949. Natural pine hybrids in Alberta. Canadian Journal of Research, Section C, Botanical Science 27: 218-229.

Newman, K.W. and R.C. Jancey. 1983. Character selection and data structure in geographic variation in *Pinus contorta*. Silvae Genetica 32: 137-141.

O'Reilly, C. and J.N. Owens. 1989. Shoot, needle, and cambial growth phenology and branch tracheid dimensions in provenances of lodgepole pine. Canadian Journal of Forest Research 19: 599-605.

Owens, J.N. and M. Molder. 1994. The reproductive cycle of lodgepole pine. British Columbia Ministry of Forests, Victoria, B.C. 35 pp.

Perry, D.A. and J.E. Lotan. 1977. Opening temperatures in serotinous cones of lodgepole pine. USDA Forest Service Research Note INT-228, Intermountain Forest and Range Experiment Station, Ogden, Utah. 6 pp.

Perry, D.A. and J.E. Lotan. 1979. A model of fire selection for serotiny in lodgepole pine. Evolution 33: 958-968.

Pfister, R.D. and J.R. Daubenmire. 1975. Ecology of lodgepole pine (*Pinus contorta* Douglas). Pp. 27-46 *in* Proceedings of Symposium on Management of Lodgepole Pine Ecosystems, Washington State University, Pullman, Washington.

Rehfeldt, G.E. 1985. Genetic variances and covariances in *Pinus contorta*: estimates of genetic gains from index selection. Silvae Genetica 34: 26-33.

Rehfeldt, G.E. 1987. Components of adaptive variation in Pinus contorta from the Inland Northwest. USDA Forest Service Intermountain Research Station Research Paper INT-375. Moscow, Idaho. 11 pp.

Rehfeldt, G.E. 1988. Ecological genetics of *Pinus contorta* from the Rocky Mountains (USA) - a synthesis. Silvae Genetica 37:131-135.

Rehfeldt, G.E. 1989. Genetic variances and covariances in freezing tolerance of lodgepole pine during early winter acclimation. Silvae Genetica 38: 133-137.

Rehfeldt, G.E., C.C. Ying, D.L. Spittlehouse and D.A. Hamilton Jr. 1999. Genetic responses to climate in *Pinus contorta*: Niche breadth, climate change and reforestation. Ecological Monographs 69: 375-407.

Richardson, D.M. and S.I. Higgins. 1998. Pines as invaders in the Southern Hemisphere. Pp. 450-473 *in* D.M. Richardson (ed.), Ecology and biogeography of *Pinus*. Cambridge University Press, Cambridge, U.K.

Satterlund, D.R. 1975. Climatic factors and lodgepole pine. Pp. 297-309 *in* Proceedings of Symposium on Management of Lodgepole Pine Ecosystems, Washington State University, Pullman, Washington.

Schopmeyer, C.S. 1974. Seeds of woody plants in the United States. USDA Agriculture Handbook No. 450, Washington, D.C. 883 pp.

Smithers, L.A. 1961. Lodgepole pine in Alberta. Canadian Department of Forestry Bulletin 127, Ottawa, Ontario. 153 pp.

Sorensen, F.C. and W.T. Adams. 1993. Self fertility and natural selfing in three Oregon Cascade populations of lodgepole pine. *In* D. Lindgren (ed.), Proceedings of Pinus contorta – From untamed forest to domesticated crop. Meeting of IUFRO WP 2.02.06 and Frans Kempe Symposium, Umeå, August 24-28, 1992, Swedish University of Agricultural Sciences, Report 11: 358-374.

Stephan B.R. 1980. Diseases, insect pests and other damage in *Pinus contorta* as an exotic species. Pp. 221-230 *in* Proc. IUFRO w.p. meeting 1980 on *Pinus contorta* provenances (S2-02-06) in Norway and Sweden. Rapp. och upps. 30, Sveriges Lantbruksuniversitet, Garpenberg.

Thomas, B.R., S.E. Macdonald, M. Hicks, D.L. Adams and R.B. Hodgetts. 1999. Effects of reforestation methods on genetic diversity of lodgepole pine: An assessment using microsatellite and randomly amplified polymorphic DNA markers. Theoretical and Applied Genetics 98: 793-801.

U.S. Soil Conservation Service. 1975. Soil taxonomy (a basic system of soil classification for making interpreting soil surveys). USDA Agriculture Handbook No. 436, Washington, D.C. 754 pp.

Wagner, D.B., G.R. Furnier, M.A. Saghai-Maroof, S.M. Williams, B.P. Dancik and R.W. Allard. 1987. Chloroplast DNA polymorphisms in lodgepole and jack pines and their hybrids. Proceedings of the National Academy of Sciences (USA) 84: 2097-2100.

Wagner, D.B., J. Dong, M.R. Carlson and A.D. Yanchuk. 1991a. Paternal leakage of mitochondrial DNA in *Pinus*. Theoretical and Applied Genetics 82: 510-514.

Wagner, D.B., Z.-X. Sun, D.R. Govindaraju and B.P. Dancik. 1991b. Spatial patterns of chloroplast DNA and cone morphology variation within populations of a *Pinus banksiana – Pinus contorta* sympatric region. American Naturalist 138: 156-170.

Wang Q., G.G. Wang, K.D. Coates and K. Klinka. 1994. Use of site factors to predict lodgepole pine and interior spruce site index in the Sub-boreal Spruce zone. Research Note No. 114, British Columbia Ministry of Forests, Victoria, B.C. 26 pp.

Wang, T., S.N. Aitken, P. Rozenberg and M.R. Carlson. 1999. Selection for height growth and Pilodyn pin penetration in lodgepole pine: Effects on growth traits, wood properties, and their relationships. Canadian Journal of Forest Research 29: 434-445.

Wang, T.L., S.N. Aitken, P. Rozenberg and F. Millier. 2000. Selection for improved growth and wood density in lodgepole pine: effects on radial patterns of wood formation. Wood and Fiber Science 32: 391-403.

Wheeler, N.C. and R.P. Guries. 1982a. Biogeography of lodgepole pine. Canadian Journal of Botany 60: 1805-1814.

Wheeler, N.C. and R.P. Guries. 1982b. Population structure, genetic diversity, and morphological variation in *Pinus contorta* Dougl. Canadian Journal of Forest Research 12: 595-606.

Wheeler, N.C. and R.P. Guries. 1987. A quantitative measure of introgression between lodgepole and jack pines. Canadian Journal of Botany 65: 1876-1885.

White, E.E., E.A. Allen, C.C. Ying and B.M. Foord. 2000. Seedling inoculation distinguishes lodgepole pine families most and least susceptible to gall rust. Canadian Journal of Forest Research 30: 841-843.

Witzell, J. and M. Karlman. 2000. Importance of site type and tree species on disease incidence of *Gremmeniella abietina* in areas with a harsh climate in northern Sweden. Scandinavian J. of Forest Research 15: 202-209.

Wright, J.W. 1962. Genetics of forest tree improvement. Food and Agriculture Organization of the United Nations, Rome. 399 pp.

Wu, H.X. and C.C. Ying. 1997. Genetic parameters and selection efficiencies in resistance to western gall rust, stalactiform blister rust, needle cast and Sequoia pitch moth in lodgepole pine. Forest Science 43: 571-581.

Wu, H.X. and C.C. Ying. 1998. Stability of resistance to western gall rust and needle cast in lodgepole pine provenances. Canadian Journal of Forest Research 28: 439-449.

Wu, H.X., F.C. Yeh, R.P. Pharis, B.P. Dancik, I.B. Jiang, I. Dymock and N.K. Dhir. 1995. Genetic parameters of greenhouse growth and performance of 2-year *Pinus contorta* ssp. *latifolia*. Scandinavian Journal of Forest Research 10: 12-21.

Wu, H.X., C.C. Ying and J.A. Muir. 1996. Effect of geographic variation and jack pine introgression on disease and insect resistance in lodgepole pine. Canadian Journal of Forest Research 26: 711-726.

Wu, H.X., F.C. Yeh, N.K. Dhir, R.P. Pharis and B.P. Dancik. 1997. Genotype by environment interaction and genetic correlation of greenhouse and field performance in *Pinus contorta*. Silvae Genetica 46: 170-175.

Wu, H.X., F.C. Yeh, R.P. Pharis, N.K. Dhir and B.P. Dancik. 2000. Study of early selection in tree breeding. Silvae Genetica 49: 152-157.

Xie, C.-Y. and C.C. Ying. 1995. Genetic architecture and adaptive landscape of interior lodgepole pine (*Pinus contorta* ssp. *latifolia*) in Canada. Canadian Journal of Forest Research 25: 2010-2021.

Xie, C.-Y. and C.C. Ying. 1996. Heritabilities, age-age correlations, and early selection in lodgepole pine (*Pinus contorta* ssp. *latifolia*). Silvae Genetica 45: 101-107.

Yanchuk, A.D., F.C. Yeh and B.P. Dancik. 1988. Variation of stem rust resistance in a lodgepole pine provenance-family plantation. Forest Science 34: 1067-1075.

Yang, R.C. 1985. Effect of fertilization on growth of 30-year-old lodgepole pine in west-central Alberta. Information Report NOR-X-268, Northern Forest Research Centre, Canadian Forestry Service, Edmonton, Alberta. 11 pp.

Yang, R.-C. and F.C. Yeh. 1993. Multilocus structure in *Pinus contorta* Dougl. Theoretical and Applied Genetics 87: 568-576.

Yang, R.-C. and F.C. Yeh. 1995. Patterns of gene flow and geographic structure in *Pinus contorta* Dougl. Forest Genetics 2: 65-75.

Yang, R.-C., F.C. Yeh and A.D. Yanchuk. 1996. A comparison of isozyme and quantitative genetic variation in *Pinus contorta* var. *latifolia* by F_{st}. Genetics 142: 1045-1052.

Yang, R.-C., N.K. Dhir, F.C. Yeh and Y. Hiratsuka. 1997. Geographic variation in susceptibility of Alberta lodgepole pine to western gall rust. Canadian Journal of Forest Research 27: 1398-1405.

Yang, R.-C., N.K. Dhir and L.K. Barnhardt. 1998. Comparative assessment of genetic variation of young high-elevation lodgepole pine for height and western gall rust resistance across two sites in Alberta. Canadian Journal of Forest Research 28: 478-484.

Yang, R.-C., Z. Ye and Y. Hiratsuka. 1999. Susceptibility of *Pinus contorta—Pinus banksiana* complex to *Endocronartium harknessii*: host-pathogen interactions. Canadian Journal of Botany 77: 1035-1043.

Ye, T.Z., R.-C. Yang and F.C. Yeh. 2002. Population structure of a lodgepole pine (*Pinus contorta*) and jack pine (*Pinus banksiana*) complex as revealed by random amplified polymorphic DNA. Genome 45: 530-540.

Yeh, F.C. and C. Layton. 1979. The organization of genetic variability in central and marginal populations of lodgepole pine *Pinus contorta* ssp. *latifolia*. Canadian Journal of Genetics and Cytology 21: 487-503.

Ying, C.C. and R.S. Hunt. 1987. Stability of resistance among *Pinus contorta* provenances to *Lophodermella concolor* needle cast. Canadian Journal of Forest Research 17: 1596-1601.

Ying, C.C. and Q. Liang. 1994. Geographic patterns of adaptive variation of lodgepole pine (*Pinus contorta* Dougl.) within the species' coastal range: field performance at age 20 years. Forest Ecology and Management 67: 281-298.

Ying, C.C., K. Illingworth and M. Carlson. 1985. Geographic variation in lodgepole pine and its implications for tree improvement in British Columbia. Pp. 45-53 *in* D.M. Baumgartner, R.G. Krebill, J.T. Arnott and G.F. Weetman, (eds.) Lodgepole pine: the species and its management. Cooperative Extension Services, Washington State University, Pullman, Washington.

Ying, C.C., C. Thompson and L. Herring. 1989. Geographic variation, nursery effects and early selection in lodgepole pine. Canadian Journal of Forest Research 19: 832-841.

Zavarin, E., W.B. Critchfield and K. Snajberk. 1969. Turpentine composition of *Pinus contorta* × *Pinus banksiana* hybrids and hybrid derivatives. Canadian Journal of Botany 47: 1443-1453.

Section 6.
Black spruce (*Picea mariana*)

1. Taxonomy and use

1.1. Taxonomy

Black spruce [*Picea mariana* (Mill.) B.S.P.], known by many alternate common names including bog spruce, swamp spruce, Canadian spruce, eastern spruce, and shortleaf black spruce (Viereck and Johnston, 1990; Alden, 1997), is one of the most common and important boreal species native to North America, especially in eastern Canada. Black spruce is one of about 40 species in the genus *Picea* of the family Pinaceae, all of which are found in cooler portions of the northern hemisphere (Farrar, 1995). Ten spruce species are native to North America (Weng and Jackson, 2000). There is no consensus among taxonomists regarding subdivision of the genus, but *Picea* is often described as having three sections (*Eupicea*, also known as *Picea* or *Morinda*; *Castica*; and *Omorika*), with black spruce generally placed among the *Eupicea* (Dallimore and Jackson, 1948; Alden, 1987). Mikkola (1969) suggested dividing the genus into only two sections, *Abies* and *Omorika*. Fowler (1983) recommended adopting Mikkola's classification, but splitting the section *Omorika* into two subsections, *Omorikoides* and *Glaucoides*, and placing black spruce into the former subsection based upon species crossability. Other examples of taxonomic classification have also been proposed for the genus (e.g., see Weng and Jackson, 2000).

Black spruce is closely related to red spruce (*P. rubens* Sarg.), a sympatric species with which it is known to naturally hybridise (Perron and Bousquet, 1997). It is also considered to be closely related to the relict European species Serbian spruce (*P. omorika* [Pančić] Purk.) (Fowler, 1980, 1983), but is taxonomically more distant from white spruce (*P. glauca* [Moench] Voss), Engelmann spruce (*P. engelmannii* Parry ex Engelm.), and Sitka spruce (*P. sitchensis* [Bong.] Carrière) (Wright, 1955), species with ranges that partially overlap (or almost overlap, in the case of Sitka spruce in Alaska) with the range of black spruce. A number of different varieties of black spruce are known (Hillier, 1981; den Ouden and Boom, 1982).

1.2. Uses

Black spruce is found on a wide range of habitats throughout its transcontinental range. Structural qualities of the wood including long fibres and comparatively high relative density, plus being relatively unaffected by insects and disease compared to other associates, make black spruce a desired species for reforestation in boreal regions. The wood is sometimes utilised for lumber products, including framing material, millwork, crating, and piano sounding boards (Alden, 1997). However, this species is most notable for the high-quality pulpwood it produces. Black spruce is the most important pulpwood species in Canada, and is also of commercial importance in the Lake States of the United States (Viereck and Johnston, 1990). Specialty products include Christmas trees, spruce gum, and essential oils for aromatherapy (Viereck and Johnston, 1990; Marles *et al*., 2000).

Marles *et al*. (2000) have described a number of traditional aboriginal uses of black spruce, based on interviews with elders; these are summarised below. Pitch was chewed as a confection, and also to

provide endurance while running, or to treat heart problems. Pitch could also be utilised to treat infected wounds. Warm gum or cone decoctions were used for stomachache and mouth infections. Ground wood or charcoal was used as baby powder. Saplings were used in making traps for various animal species. The wood was used in making bark canoes, shelters, snowshoes, dolls, baskets, and used for firewood. Other uses of this species by aboriginal peoples include making fish traps and drying racks (Parish and Thomson, 1994).

2. Natural distribution and migrational history

2.1. Natural distribution

Black spruce is widespread throughout northern North America, with a mainly contiguous range (Figure 1). The natural distribution of this species extends from Newfoundland westward to British Columbia and Alaska. The range extends northward to the treeline above the Arctic Circle at 68°N latitude (Heinselman, 1957), where growth form may be adversely affected (Lavoie and Payette, 1992). The most southerly population is an isolated relict bog at Bear Meadows in central Pennsylvania at a latitude of 40°48'52"N (Abrams *et al.,* 2001). This species grows at elevations ranging from sea level along the Atlantic and Hudson Bay coasts to 1,830 m in the Rocky Mountains (Viereck and Johnston, 1990). While covering a similar natural range to that of white spruce, black spruce may be found on sites that are too extreme for the former to tolerate.

2.2. Evolution and migrational history

Li (1953) suggested that most coniferous genera originated around the periphery of the Pacific basin. Wright (1955) concurred, and proposed that *Picea* most likely originated in northeastern Asia. Florin (1963) inferred from fossil records that generic divergence within the Pinaceae took place about 135 million years ago. There is lack of agreement on whether *Picea* is more closely related to *Pinus* or to *Pseudotsuga* (e.g., Florin, 1963; Prager *et al*., 1976; White *et al.,* 1993), but Prager *et al*. (1976) suggested that *Picea* may have been one of the first genera to emerge. However, the genus *Picea* has remained evolutionarily conservative (Wright, 1955; Ogilvie, 1972), with all members having a haploid number of 12 chromosomes and most species of the genus having similar karyotypes (Roche and Fowler, 1975).

Conventional theory holds that the entire genus migrated to North America across the Bering Sea *via* a land bridge connection between Siberia and Alaska (Hills and Ogilvie, 1970; Nienstaedt and Teich, 1971). Wright (1955) proposed that black spruce and red spruce migrated independently to North America in an earlier migration than that which brought the northwestern spruces to North America. Fowler (1980) hypothesised that red spruce might have arrived in eastern North America from Europe prior to continental separation during the Cretaceous or early Tertiary Periods, while black spruce arrived in Alaska from Siberia. He believed that Serbian spruce was ancestral to both black and red spruce. An investigation of ribosomal DNA sequences has lent support to the theory that red and black spruce migrated independently from other North American spruces and are closely related to Serbian spruce (Smith and Klein, 1994). Hills and Ogilvie (1970) described fossilised cones from an ancestral species (*Picea banksii*) found in the Canadian Arctic which they believed to be an evolutionary link between Eurasian and North American species, although this theory has not been universally accepted (Roche and Fowler, 1975). Recent evidence points to the possibility that red spruce was derived from fragmentation of a large black spruce population during the Pleistocene era, with subsequent allopatric speciation resulting from genetic drift (Perron *et al.,* 2000; Jaramillo-Correa and Bousquet, 2003).

Figure 1. Natural distribution of black spruce in North America



Source: US Geological Survey 2002, online at: http://climchange.cr.usgs.gov/data/atlas/little/; after Little, 1971

The earliest spruce pollen fossils, dating back to 70 million years before present (b.p.), have been found in North America and elsewhere, according to Morgenstern and Farrar (1964). They noted that fossils resembling modern spruce species, located in Europe, date back to 13 million b.p. These authors stated that black spruce fossil records date back to 1 million b.p.; evidence suggests that the species has been in eastern North America for at least 100,000 years.

A general period of cooling prior to the onset of the last glacial advance, beginning about 25,000 b.p., caused the southward retreat of coniferous forests. There is evidence to suggest that black spruce was able to tolerate the cooling conditions longer than most other associated species. Pollen records from the US Midwest, where *Picea* and *Pinus* forests had predominated in the region

prior to the ice age, showed that *Pinus* was gradually replaced by *Picea*, and black spruce became more common than white spruce, during the cooling period prior to glaciation (Baker *et al.,* 1989). By 22,700 b.p. all spruce pollen percentages began to decline. The last (Wisconsinian) glaciation culminated about 18,000 b.p. (Conkle, 1992), when the Laurentide ice sheet was estimated to extend south of the 40th parallel in eastern North America (Critchfield, 1984; Andrews, 1987), and boreal species from present-day northeastern North America were centred in the southeastern US (Webb *et al.,* 1987). Pollen records indicate that spruce was centred south of the Laurentide ice sheet, below the Great Lakes, with less abundant populations extending eastward below the southern margin of the ice sheet (Jacobson *et al*., 1987). Recent mitochondrial DNA analyses have pointed to three or four refugia for black spruce: one west of the Rocky Mountains, probably south of the Cordilleran ice sheet in Washington or Oregon; one near the Great Lakes, west of the Mississippi Valley; one in the New York / New England / central Appalachian Mountains region; and possibly a fourth refugium in unglaciated regions of Labrador and Newfoundland (Jaramillo-Correa *et al.,* 2004).

Following deglaciation, northward migration of *Picea* from its southerly refugia took place. Fossil records show that spruce-dominant forests were established in Manitoba by 11,600 b.p., and in Alberta by 9,800 b.p. (Ogilvie, 1972). In northern Québec near the Hudson Bay coast, black spruce was the first species to colonise the area immediately after deglaciation around 6,000 b.p. (Desponts and Payette, 1993). At the boreal forest - shrub-tundra transition zone in northwestern Québec, black spruce was the first coniferous coloniser, and upon establishment, has dominated the region since 4,000 b.p. (Gajewski *et al.,* 1993). This concurs with Morgenstern and Farrar (1964), who listed resistance to low temperatures, year-round seed dispersal, and having the ability to reproduce by layering should temporary cooling occur as traits ensuring that black spruce was particularly well adapted for northern migration compared to other associated species. Maps of inferred spruce migration from 18,000 b.p. to present are found in Webb *et al.* (1987).

3. Reproductive biology

3.1. Reproductive bud differentiation

In *Picea*, bud differentiation occurs between mid- to late July, when shoot elongation is almost complete (Owens and Blake, 1985). Reproductive bud development continues until dormancy, and continues again the following spring. Timing of floral receptivity may vary by a few weeks according to spring weather conditions, but commonly occurs around mid-May to early June in the southern portion of the range, and a few weeks later in the north (Vincent, 1965; Viereck and Johnston, 1990). The seed cone, which remains receptive for about 10 days, is well-synchronised with peak pollen shedding (Ho, 1991). Ovuliferous scales funnel pollen to the micropylar arms of the ovule, where a pollination drop carries pollen into the pollen chamber (Ho, 1991). Female gametophyte development proceeds throughout the summer. Cones mature in late August to early September, 3 months after pollination; seed dispersal begins in October (Safford, 1974; Young and Young, 1992).

3.2. Natural seed production and dissemination

Black spruce female ovulate strobili are usually located at the top of the crown near the shoot terminals. Male strobili may be well distributed throughout the crown, but are typically found in the lower $^{2}/_{3}$ of the crown (Ho, 1991). Seed cones have been observed on black spruce as young as 6 or 7 years of age (Morgenstern and Fowler, 1969; Caron and Powell, 1992), with seed cones common on 10 to 20-year-old trees (e.g., Heinselman, 1957; Horton and Lees, 1961; Vincent, 1965). Cone production increases with tree age (Skeates and Haavisto, 1987; Caron and Powell, 1989), to an optimal age that ranges from 50 to 150 years (Heinselman, 1957). Black spruce is one of the least periodic of spruces, and may produce successive seed crops for many consecutive years (Park *et al.,* 1998), with heavy crops occurring at about 2 to 6-year intervals (Vincent, 1965; Safford, 1974;

Caron and Powell, 1992). Initial pollen cone production may lag behind that of seed cones by several years (Simpson and Powell, 1981; Caron and Powell, 1989).

Black spruce cones are persistent and semi-serotinous. Cones can persist on the tree for more than 20 years (Horton and Lees, 1961; Atkinson and Haavisto, 1992), and viable seed within cones has been found after 25 years (Safford, 1974; Viereck and Johnston, 1990). However, viability of seed within cones begins to drop after 3 to 4 years (Haavisto, 1975), decreasing to very low levels after 15 to 20 years (Chai and Hansen, 1952; Waldron, 1957 as cited by Horton and Lees, 1961; Haavisto, 1975). In the absence of fire, the cones open slowly and may disperse seed for several years after ripening (Chai and Hansen, 1952; Johnston, 1977). Seed dispersal can occur throughout the year (Heinselman, 1957; Vincent, 1965), with peak dispersal reported from July to August in Newfoundland (Howard, 1962, as cited in Vincent, 1965), during July in Alaska (Zasada *et al*., 1979), and March to April in Ontario (Haavisto, 1978). Seed may remain viable in the seedbed for one to two years after dispersal (MacGillivray, 1955; Fraser, 1976; Thomas and Wein, 1985). Forest fires may open closed cones on standing trees, significantly increasing seed dispersal in subsequent months or years (Wilton, 1963; Zasada *et al*., 1979). Because of their semi-serotinous characteristic, even scorched cones of fire-killed trees may retain viable seed (Horton and Lees, 1961). Germinative capacity, however, of both dispersed seed and seed remaining in cones can be reduced by fires of sufficiently high intensity (Zasada *et al.,* 1979). Simpson *et al*. (2004) described high germinability (above 80%) of black spruce seed stored for 30 years under ideal conditions (-20˚C and moisture content between 5 and 8%), which they attributed to the semi-serotinous nature of the cones. These authors inferred that seed of this species kept under ideal conditions in undisturbed sealed containers could have a potential storage longevity of up to 100 years.

3.3. Natural regeneration

3.3.1. Seedling regeneration

Despite its very small seed, black spruce exhibits good germination and establishment on many sites. Sphagnum mosses may provide ideal seedbeds, as they retain moisture and provide aeration (Place, 1955). However, if the sphagnum grows too rapidly, it may engulf the black spruce germinant, preventing establishment (LeBarron, 1948; Roe, 1949). Feathermosses provide an adequate seedbed during wet years if not too dense, but may dry out prior to seedling root penetration (Viereck and Johnston, 1990). Litter seedbeds may also prevent root penetration; Place (1955) suggested that the best litter seedbeds are composed of a variety of materials. Moist decayed wood is a favourable substrate (Vincent, 1965). Upland exposed mineral soils, particularly sandy loams, may be suitable, although they are sometimes prone to waterlogging, frost heaving, or drying (Place, 1955; Vincent, 1965). Light burns may favour regeneration of competing species, but severe burns, in which the subsequent organic matter layer is thin or absent, favour black spruce establishment (Vincent, 1965). However, after fire, the burned surfaces may be too hot to allow establishment (LeBarron, 1944; Place, 1955). Manganese in the humus of drier soils is toxic to black spruce seedlings (Duchaufour and Rousseau, 1959).

Germination is epigeal (Safford, 1974). Cotyledon number varies, with 4 commonly being observed (Place, 1955). Survival is highest when germination takes place in June (Heinselman, 1957). Following a winter harvest and prescribed burn in Alberta, seventy percent of germination occurred in June; overwinter survival of June germinants was greater than that of July or August germinants (Berger and Gilmore, 2003). In the first year, early summer rainfall is required. Although initial establishment may be better under light cover than in the open, subsequent survival and growth are better on open sites (LeBarron, 1944; Place, 1955); as black spruce flushes so late, risk of frost damage is minimal. Upon germinating, roots penetrate the soil to about 1 cm (Heinselman, 1957). On upland soils, roots may penetrate to 5 cm after the first growing season, but on moss seedbeds,

roots may only extend 3.5 cm after 2 years (Fowells, 1965; Viereck and Johnson, 1990). On some sphagnum bogs where growing moss buries seedling roots and the groundwater is rising, black spruce seedlings may respond by producing advantitious roots from stems (Fowells, 1965). Mycorrhizae are abundant on most sites except those with sphagnum (Place, 1955).

During the first year, seedlings grow only a few cm tall in open sites, less in the understory (LeBarron, 1944). Early growth is often slower on mineral soil than on duff. After 3 years, height ranges from 7 cm to 45 cm on the best sites (Heinselman, 1957). Beyond the first 3 or 4 years, juvenile growth patterns begin to exhibit wide variations associated with habitat; in open, productive upland sites, seedlings may grow 15 to 25 cm a year; on poor muskegs, annual growth may only be 2.5 cm (Heinselman, 1957).

3.2.2. Vegetative propagation

Black spruce may reproduce vegetatively through layering, a process by which live branches at the base of the stem become embedded in suitable rooting medium, such as moss or a thick organic layer, forming adventitious roots and eventually an independent stem (Stanek, 1961). Layering occurs across the range of the species (Stanek, 1975). It may be an important form of regeneration in areas where conditions are not favourable to regeneration by seed (Stanek, 1961; Legere and Payette, 1981) and where fire is less frequent or intense (Richardson, 1981). However, well-stocked even-aged stands having a dense canopy may not produce many layers, as the stems self-prune and do not maintain live branches to the ground (Johnson, 1956; Stanek, 1961).

Stems of layer origin respond favourably to release by cutting, and may be an important source of advanced growth and regeneration (Paquin and Doucet, 1992; Sims and Walsh, 1995). Black spruce of layer origin may grow as well or better than those of seedling origin (Stanek, 1961; Vincent, 1965), though layers, particularly younger stems, may initially have poorer, curved stem form (Stanek, 1968; Foster, 1985). Paquin *et al.* (1999) found that natural seedlings had a greater annual height growth than layers for the first 8 years after release, but equivalent growth rates in years 11 to 15, suggesting a period of acclimation was required by the layers. Nevertheless, layering can produce trees of merchantable size (LeBarron, 1948; Stanek, 1961, 1968). Trees of layer origin may have height, diameter and merchantable volume comparable to those of seedling origin (Lussier *et al*., 1992; Morin and Gagnon, 1992).

3.4. Mating system and gene flow

Black spruce is monoeceous, with a mixed mating system. While mainly outcrossing, self-pollination also occurs. Numerous studies from throughout the species range, investigating variation among populations in allozymes, random amplified polymorphic DNA (RAPD), or sequence-tagged-site (STS) markers from populations separated by between 2 km to sampling the entire range, have found that most of the total genetic variation available resides within populations. Similar estimates of 1% of the total genetic variation attributed to among-population differences were reported for New Brunswick (Boyle and Morgenstern, 1987), Québec (Isabel *et al.,* 1995; Perry and Bousquet, 2001), Ontario (Boyle *et al.,* 1990), and Alberta (Wang and MacDonald, 1992). Perry and Bousquet (2001) found complete outcrossing in both seed-origin and asexual layering-origin stands. Slightly higher estimates of 6% among-population variation were estimated for Newfoundland (Yeh *et al.,* 1986), for isolated stands near the northern species limit in subarctic Québec (Desponts and Simon, 1987), and in northern Ontario (O'Reilly *et al.,* 1985). While some of the above studies were based on low numbers of sampled populations or loci assessed, they do point to widespread outcrossing in black spruce.

Other studies have shown evidence of somewhat higher rates of self-fertilisation in black spruce. Average isozyme multilocus outcrossing rates of 0.924 in New Brunswick (Boyle and Morgenstern,

1986), 0.621 in Alberta (Sproule and Dancik, 1996), and 0.837 for a clonal seed orchard in Ontario (Barrett *et al.,* 1987) were obtained. Park and Fowler (1984), in a study of controlled pollinations in a natural black spruce stand, found a self-fertility rate of 47.2%. Morgenstern (1972) sampled 3 populations each from southern and northern Ontario, and found average inbreeding coefficients of 0.08 for southern populations and 0.03 in the north.

Population substructuring in black spruce appears minimal to nonexistent (Boyle and Morgenstern, 1986, 1987; Knowles, 1991; Perry and Bousquet, 2001). Clustering of similar genotypes was observed in one lowland stand, but mature trees in that stand were not inbred, although trees from an upland stand did exhibit some inbreeding (Boyle *et al.,* 1990). Factors which may prevent significant neighbourhood structure include simultaneous receptivity (O'Reilly *et al.,* 1982), effective gene migration, and absence of diversifying selection intensities over homogeneous environments (Boyle and Morgenstern, 1984).

Sampled Québec populations were in Hardy-Weinberg equilibrium (Isabel *et al.,* 1995). However, investigations of black spruce seed orchard crops, plantations, and natural stands have determined that mating is oftentimes not random, and frequently there are unequal parental contributions to the seed crop (O'Reilly *et al.,* 1982; Knowles, 1985; Barrett *et al.,* 1987; Caron and Powell, 1989; Rogers and Boyle, 1991; Perry and Bousquet, 2001). Particularly in young orchards that are just reaching sexual maturity, a few parents may be the major contributors to the gene pool. Flowering phenology does not appear problematic (O'Reilly *et al.,* 1982), as the typical black spruce habitat experiences a relatively short spring warmup period and short pollination periods, and hence flowering is largely synchronous within a region. Rogers and Boyle (1991) found differences in male reproductive success that could not be attributed to pollen viability. They postulated a number of potential causes: male competition, such as in rate of pollen tube growth; female selection; or post-fertilisation embryo abortion.

Black spruce pollen and seed are both windborne. Typical seed and pollen dispersal for wind-pollinated species generally shows strongly leptokurtotic, skewed distributions (Ellstrand, 1992), with the majority of both pollen and seed remaining close to the parent. However, the accumulated effect of small amounts of pollen from many parents dispersing over long distances can be considerable (Adams, 1992). While seed appears to travel approximately the same distance (Adams, 1992) or slightly less far than pollen (Ellstrand, 1992) for those anemophilous species investigated, because seed is diploid, it has twice the influence on effective population size than the equivalent pollen dispersal (Adams, 1992). Seed is also a better indicator of gene flow than pollen, as it is more likely to produce progeny. As well as seed and pollen dispersal, gene flow depends upon pollen viability, flowering synchrony, successful fertilisation, cone abortion rates, seed viability, seed germination, and seedling establishment. Wind patterns and precipitation during pollen flight may affect pollen dispersal distances, while additional factors influencing seed dispersal include air currents, stand structure and forest fragmentation, and availability of suitable habitat for germination.

Gene flow is thought to be relatively high for most conifer species, including black spruce, at least throughout most of its range. Black spruce pollen dispersal is aided by sacci wings (Owens and Blake, 1985). Black spruce bears the smallest seed of any North American *Picea*, with about 890,000 seeds per kg (Viereck and Johnston, 1990). Thus both seed and pollen are capable of travelling long distances. The longevity, large population size, continuous geographic distribution, high rate of outcrossing, and minimal barriers to gene flow all contribute to effective gene flow in this species. Govindaraju (1988) assessed gene flow based on numbers of migrants per generation, and rated black spruce as having high gene flow. In examining the genetic structure of five isolated black spruce stands located on a chain of small islands in subarctic Québec, Desponts and Simon (1987) suggested that gene flow between the populations was sufficiently high enough to override the effect of geographic isolation. Populations near the northern treeline in Québec exhibited high levels of diversity in nuclear DNA, but not in maternally-inherited mitochondrial DNA, suggesting slower seed

dispersal compared to pollen dispersal (Gamache *et al.,* 2003). These authors speculated that vegetative layering in populations near the northern extreme of the species range may actually help to maintain long-term genetic diversity during periods when climatic conditions were less favourable for seed production in these regions.

Viereck and Johnston (1990) stated that black spruce seed dispersal is effective up to 79 m from the windward edge of a stand. Because seed can be released at any time of year, it has the potential to travel more than a mile over crusted snow (Heinselman, 1957); postfire opportunities allow for greater gene flow through reduction of mating barriers and release of seed from the opening of semi-serotinous cones. Pollen of this species has been inferred to travel at least 3.5 km downwind. A rogued black spruce seed orchard ($^2/_3$ of trees removed), coupled with a subsequent decrease in orchard pollen relative to contaminant pollen, resulted in 35% more pollen contamination than in an adjacent unrogued orchard, the source of foreign pollen which the author attributed to a stand located 3.5 km upwind of the orchard (Caron, 1994). A recent study found samples of black spruce pollen 300 m above the ground during maximal pollen release (Di-Giovanni *et al.,* 1996). The authors surmised that, based on grain weight, the pollen could potentially drift 47 km from its source given a steady windspeed of 5 m s^{-1}.

Gene flow between red spruce and black spruce was initially described as minimal, with no real directional difference in crossability (Gordon, 1976). Barriers to gene flow between these species would be expected, as hybrids do not appear to be superior to either parental species, with the exception of resistance to winter desiccation**.** Hybrids were less fit in tolerance to wet sites than black spruce, and less fit in shade tolerance to hardwood competition than red spruce, indicating presence of strong selection pressures against species introgression. However, later studies have found high amounts of gene flow between these two species, with no asymmetric directionality (Perron and Bousquet, 1997).

4. Hybridisation

A number of artificial crosses have been carried out between black spruce and other *Picea* species. Black spruce is most easily crossed with Serbian spruce (Gordon, 1976; Fowler, 1980), which is generally accepted as taxonomically being the most closely related species to black spruce. Artificial crosses have been made with Sitka spruce (Fowler *et al*., 1979; Gordon, 1981; Fowler, 1983), a west coast species that approaches but does not overlap the range of black spruce. A putative natural black × Sitka spruce hybrid was located in Alaska (Gordon, 1985). Crosses have also been made with Engelmann spruce (Fowler *et al*., 1979), whose range slightly overlaps that of black spruce in southern British Columbia. Apparent crosses have also been made with blue spruce (*P. pungens* Engelm.); Norway spruce (*P. abies* [L.] Karst.) of northern Europe; Yeddo spruce (*P. jezoensis* [Sieb. and Zucc.] Carr.), Sakhalin spruce (*P. glehnii* [Fr. Schmidt] Masters.), and Koyama spruce (*P. koyamai* Shiras.) of Japan; Oriental spruce (*P. orientalis* [L.] Link.); Korean spruce (*P. koraiensis* Nakai); Mexico spruce (*P. mexicana* Martinez); dragon spruce (*P. asperata* Mast.); and Himalayan spruce (*P. smithiana* [Wall.] Boiss.) (Wright, 1955; Morgenstern and Fowler, 1969; Fowler *et al.,* 1973; Gordon, 1975, 1976, 1985).

Although their ranges are largely sympatric, white and black spruces do not readily hybridise. Putative natural hybrids between these species based on intermediate morphology have been observed in northern British Columbia (Roche, 1968, 1969), near the treeline in the Northwest Territories (Larsen, 1965), and in eastern Manitoba (Dugle and Bols, 1971). However, in harsh northern environments, it may be very difficult to distinguish the two species from each other, making identification of possible hybrids based on morphology imprecise.

Much attention has been focussed on a single tree from Minnesota which is believed to be a first-generation hybrid of white × black spruce. Named the Rosendahl spruce, this tree, growing in an abandoned pasture, displays intermediary morphological characteristics (Little and Pauley, 1958), leaf oil terpene composition (von Rudloff and Holst, 1968), flavonoid compound content (Riemenschneider and Mohn, 1975), vegetative and sexual bud size (Winton, 1964c), and heat sum requirements for both initiation of meiosis and pollen release (Winton, 1964a) between those of white spruce and black spruce. However, the validity of the Rosendahl spruce as a hybrid was questioned by Gordon (1976). A study using aggregate hybrid indices for numerous cone and twig characters resulted in the Rosendahl spruce being grouped with white spruce (Parker and McLachlan, 1978). A recent investigation using inter-simple sequence repeat (ISSR), RAPD, and cytological markers on open-pollinated seed collected from the Rosendahl spruce confirmed that the putative hybrid is most likely a white spruce genotype (Nkongolo et al., 2005).

Artificial crosses between black spruce and white spruce are difficult to produce (Fowler *et al*., 1971; Rauter, 1971; Gordon, 1979; Fowler, 1983). Wright (1955) claimed to have produced small quantities of hybrid seed, but the hybridity of the cross was unverified. Fowler *et al.* (1979) were successful at crossing black spruce from New Brunswick with white spruce from British Columbia, suggesting that barriers to crossing are most evident where the ranges of the two species are sympatric.

The low incidence of natural hybrids and lack of ease in artificially crossing white and black spruce led to speculation regarding barriers to crossing. Winton (1964a) postulated that the difference in heat sum requirements, leading to differential timing of pollen release in these species, is probably the primary isolating mechanism. The frequency of hybridisation between sympatric species tends to be highest near the range limits and / or on disturbed sites where plant competition is less.

Hybridisation between red spruce and black spruce has been extensively studied. Artificial crosses have been made in numerous locations. Natural hybrids between these species occur where their ranges coincide. Hybrid swarms are typically found on sites that have been repeatedly or severely disturbed, where competition is lessened and remnant red spruce trees will be flooded by the more abundant black spruce pollen. In these situations, selection against hybrids appears density-dependent (Manley and Ledig, 1979).

Because of the phenotypic similarity of red and black spruce, much emphasis has been placed on developing definitive methods to distinguish these two species. Techniques include morphological trait differences (Jablanczy, 1964; Morgenstern and Farrar, 1964; Manley, 1971; Gordon, 1976; Weng and Jackson, 2000) and isozyme analysis (Eckert, 1989; Hawley and DeHayes, 1994). Pure red spruce and especially black spruce stands both have relatively high levels of variation, causing subjectivity in interpreting the observed variation using hybrid indices based on morphology. The advent of molecular techniques has allowed for more efficient and sensitive estimates of hybridisation and introgression. Molecular approaches have included restriction fragment length polymorphisms (RFLP's) (Bobola *et al.,* 1992), RAPD's (Perron and Bousquet, 1997; Nkongolo *et al*., 2003), nuclear and chloroplast single nucleotide polymorphisms (SNP's) (Germano and Klein, 1999), and single sequence repeats (SSRs) (Campbell *et al*., 2005).

Estimates of the degree of hybridisation and introgression between black and red spruce vary widely. Widespread hybridisation between red and black spruce throughout the sympatric portion of their ranges has been reported (Morgenstern and Farrar, 1964; Manley, 1972; Perron and Bousquet, 1997). Morgenstern and Farrar (1964) and Perron and Bousquet (1997) described the occurrence of introgression between the two species. Morgenstern and Farrar (1964) predicted that hybrids would subsequently backcross with parental types, which are more fertile than the hybrids, rather than crossing amongst themselves. Alternatively, Manley (1972), Gordon (1976), and Hawley and DeHayes (1994) found little evidence for introgression in parental stands.

Berlyn *et al.* (1990) detected hybridisation along elevational gradients between red spruce and relict black spruce that was occupying higher elevations in mountainous regions of New England. They also noted an east-west gradient, with black spruce proportionally more abundant in the eastern portion of the sympatric region. However, Bobola *et al*. (1996) and Hawley *et al.* (2000) did not observe strong elevational clines in these mountain ranges, nor did they observe black spruce to be dominating high-elevation sites. Morgenstern and Farrar (1964) hypothesised that introgression is strongest at the limits of a species' range, where populations are reduced in vigour (in Nova Scotia for introgression of red spruce genes into black spruce, and in Québec at the northwestern limit of the red spruce range for introgression of black spruce genes into red spruce populations). Small amounts of introgression have been identified in allopatric populations of the species (Eckert, 1989; Bobola *et al*., 1996; Perron and Bousquet, 1997), suggesting the possibility of historical hybridisation.

Timing of flowering is not asynchronous in red and black spruce and thus not a barrier to crossing. Gordon (1976) observed numerous dead hybrid embryos in artificial crosses between the two species, and ascribed his results to embryo inviability. Negative heterosis of photosynthetic rate was apparent in young red × black spruce hybrid seedlings (Manley and Ledig, 1979), but had disappeared by age 22 in these same hybrid families (Johnsen *et al*., 1998). If, as theorised by Morgenstern and Farrar (1964) and Perron and Bousquet (1997), red and black spruce were previously geographically isolated prior to 1 million years b.p., they may have undergone reproductive isolation and divergent evolution, with black spruce evolving in a boreal climate and red spruce evolving under cool temperate conditions. With the glacial retreat, opportunities for secondary contact and subsequent hybridisation occurred during northward migration, preventing further divergent evolution between the two species. RAPD fingerprints have confirmed the close phylogenetic affinity between red spruce and black spruce (Perron *et al.,* 1995).

Within black spruce, interprovenance hybrid vigour was apparent in 10-month-old seedling growth, at least for crosses made between black spruce populations from eastern Canada, but hybrid superiority was less evident by age 5 (Morgenstern, 1974b, 1975a). However, no evidence for, or slightly negative, heterosis was inferred for ability to withstand winter desiccation in the same test populations.

Major *et al.* (2003) observed negative heterosis in height growth of mature hybrid red × black spruce trees, but not in hybrid seedlings. Major *et al.* (2005) proposed that ecophysiological and crossability barriers (e.g., low proportion of filled seeds of hybrids) may be more important than severe negative heterosis for maintaining species integrity.

5. Genetics

5.1. Cytology

In black spruce, as with all members of the genus *Picea*, the diploid (2*n*) number is 24, and karyotypes are similar to others in the genus (Morgenstern, 1962; Roche and Fowler, 1975). The karyotype is asymmetrical, and considered semi-advanced. Of the 12 chromosome pairs, 3 have submedian centromeres, one is median-submedian, six are median, and two are metacentric; five of the chromosome pairs have a distinctive secondary constriction on one of their arms (Nkongolo and Klimaszewska, 1993). Although black spruce chromosomes are visually similar to those of red spruce, the latter species has been found to contain about twice as much nuclear DNA as black spruce, which averaged only 24 picograms (Berlyn *et al.,* 1990). As with many other gymnosperms, the chloroplast of black spruce appears to be paternally inherited, while the mitochondria are maternally inherited (Bobola *et al.,* 1996).

Winton (1964b) observed that about 1 in 23,000 black spruce seedlings were tetraploid (4*n*), apparently from chromosome doubling in proembryo initials. Such polyploid seedlings were stunted,

with short, thick needles, and would probably not survive unless protected from plant competition. Sectional chimeras containing both 2*n* and 4*n* tissue on the same plant have also been found (Winton, 1964b).

5.2. Inbreeding depression

The estimated number of embryonic lethal genes in black spruce is 5 to 7, which is lower than for some other conifers (Park and Fowler, 1984). While these authors found the effect of the genetic load on germination rates to be relatively low in their study, survival of 2-year-old selfed seedlings was only 75% that of progeny arising from a polymix. Others have also found evidence of inbreeding depression. Morgenstern (1972) found lower percentage filled seed and germination plus higher percentage chlorophyll-deficient seedlings in southern compared to northern Ontario populations. He hypothesised that inbreeding in southern populations was a result of those populations being more isolated and smaller in number than the northern ones sampled.

In a study involving a complete 7x7 diallel, Morgenstern (1974a) observed inbreeding depression in germination, survival, and height to age 2; severe inbreeding depression in the form of much reduced growth was still evident in these selfed trees at age 14 (Boyle, 1987). Sampling the same diallel material in a later study, Johnsen *et al*. (1999) found that inbreeding greatly depressed growth, but not carbon isotope discrimination, and stated that selfing did not disrupt photosynthetic potential, but did appear to disrupt subsequent physiological processes that contributed to growth. Further investigation of this study material by Johnsen *et al.* (2003) led to the finding that while carbon fixation did not differ between selfed and outcrossed progeny, utilisation of fixed carbon is apparently modified in the surviving selfed progeny.

Sproule and Dancik (1996) discovered that multilocus outcrossing rates of seeds from cones that had been retained for many years on the tree were significantly higher than the rates for the youngest cones. Their results suggest pregerminative selection against selfed seeds. Whereas Morgenstern (1974a) stressed the importance of high levels of heterozygosity for pioneer species such as black spruce which tolerate suboptimal environments, Sproule and Dancik (1996) pointed out that self-compatibility may be desirable for colonising new habitats.

5.3. Genetic variation

5.3.1. Population-level variation

Range-wide black spruce provenance studies have indicated that clinal variation is the predominant pattern of variation to emerge, particularly in growth and survival at an early age (Morgenstern and Mullin, 1990). The main processes shaping north-south gradients are photoperiod and temperature-based (e.g., growing degree-days, length of growing season). Traits showing clinal variation include germination, growth, juvenile indeterminate shoot expansion, phenology, drought resistance, hardiness, survival, and wood density (Pollard and Logan, 1974; Khalil and Douglas, 1979; Segaran, 1979; Fowler and Park, 1982; Bihun and Carter, 1983; Boyle, 1985; Khalil, 1985; Beaulieu *et al.,* 1989; Morgenstern, 1996). Whereas best growth generally occurs in provenances from regions with more degree-days, best survival occurs with fewer degree-days; thus height and survival trends in relation to growing degree-days are negatively correlated, and fastest-growing provenances often do not have best survival rates (Boyle, 1985; Morgenstern and Mullin, 1990). Clinal variation lessened as seedlings aged in both Ontario and Québec (Boyle, 1985; Beaulieu *et al.,* 1989), but clinal patterns had not diminished up to age 15 in Newfoundland (Hall, 1986). In north-central Alberta, where the Rocky Mountain range has a major impact on the geographic landscape, growth varies clinally with elevation and precipitation (Wei *et al.,* 2004).

One exception to the general clinal pattern of variation occurs on the island of Newfoundland. At age 4, variation appeared ecotypic (Khalil, 1975). While weak north-south trends were noted in central Newfoundland at ages 10 (Khalil, 1986) and 15 (Hall, 1986), growth at age 15 remained ecotypic in the northwestern and southeastern peninsulas of the island, although no trends were noted for survival (Hall, 1986). Complex climatic patterns occur in Newfoundland due to maritime influences, including the cold Labrador current bringing Arctic sea ice and delaying air temperature warming in spring, and summertime southwest winds which cause summer temperatures to be cooler in the south than in the north (Khalil and Douglas, 1979). It has been suggested (Boyle, 1985) that glacial refugia for black spruce may have occurred in Newfoundland, with subsequent random drift and selection processes resulting in genetic differentiation from mainland populations.

Monoterpene composition also does not conform to clinal patterns of variation. Chang and Hanover (1991) separated range-wide populations into 2 east-west clusters, with the dividing line east of the Ontario-Manitoba border. The authors suggested that the clusters represented a branching of populations during the last glaciation.

Numerous investigations exploring potential differences between upland and lowland stand types have been carried out; most found minor or no evidence of edaphic ecotypes (e.g., Morgenstern, 1973, 1996; Fowler and Mullin, 1977; Boyle *et al.,* 1990; Thomson *et al.,* 1990; Wang and MacDonald, 1992, 1993; Zine El Abidine *et al.,* 1994a, 1994b, 1995), although O'Reilly *et al.* (1985) did note greater isozyme differentiation in upland compared to lowland sites. Gene frequency differences would not be expected because of high migration between stands, and low likelihood of black spruce regeneration on upland sites in the absence of disturbance. It is therefore unlikely that adaptation to upland sites would have evolved (Fowler and Park, 1982).

Black spruce generally has high levels of genetic variation for many traits, not unexpected for a hardy species with a widespread, continuous range and adequate gene flow that is adapted to numerous site types. Range-wide provenance studies revealed that best growth occurs at locations around the Great Lakes, with poorest growth at northern sites such as those in northern Québec having cold, nutrient-poor soils and short growing seasons (Morgenstern and Mullin, 1990). Survival was lowest in harsh northern Ontario sites, and highest at the Newfoundland and Prince Edward Island tests.

Typically, better growth at a location occurred with southerly provenances transferred northward, while in boreal regions, southward transfer increased survival (Morgenstern and Mullin, 1990). Local provenances did not always perform the best at a site (Fowler and Park, 1982; Hall, 1986). Poorest performance tended to be with sources from the range extremes (Bihun and Carter, 1983; Boyle, 1985). While provenance × environment interactions occurred at most locations, they were not evident in the Maritimes (Fowler and Park, 1982) or in 15-year survival in Ontario (Boyle, 1985). Observed provenance rank changes and poor age-age correlations in Newfoundland, the Maritimes, the Lake States, Québec, and Ontario led investigators to recommend not practicing early selection in this species based on young seedling measurements (Nienstaedt, 1984; Boyle, 1985; Hall, 1986; Park and Fowler, 1988; Beaulieu *et al.,* 1989). One explanation may be that taller seedlings undergo more planting shock, leading to rank switches (Beaulieu *et al.,* 1989). Juvenile-mature phenotypic correlations of wood ring width at dbh are unreliable until around age 20 (Koubaa *et al.,* 2000).

5.3.2. Within-population variation

High levels of genetic variation in growth and survival of black spruce have been documented in numerous studies. Table 1 lists a sampling of individual and family heritabilities reported for height growth of black spruce across a number of test sites.

Slow-growing black spruce families, including selfed families, appear to have lower endogenous concentrations of growth-promoting gibberellins (Pharis *et al.,* 1991). While predetermined shoot expansion of young black spruce seedlings varies little, genotypes with more indeterminate growth prior to the time when all growth becomes virtually predetermined (between age 5 to 10) has been suggested as a mechanism conferring an early growth advantage that may persist beyond this period (Pollard and Logan, 1974). Mullin *et al.* (1995) suggested that by using short-term testing with extended growth cycles which maximise the opportunity for free growth, culling decisions on a family basis may be made after 4 growth cycles. Trees that flush earlier, with a lower accumulated heat sum threshold, have more leader extension (O'Reilly and Parker, 1982).

Table 1. Examples of reported black spruce individual (h^2_i) and half-sib family (h^2_f) heritabilities for height across multiple locations

Reference	location	# families	# sites	age	h^2_i	h^2_f
Mullin *et al*., 1995	N.B. (1979 series)	30	6	5	0.2	0.8
″	″	″	″	10	0.3	0.9
″	″	″	″	15	0.3	0.9
″	N.B. (1980 series)	45	6	5	0.1	0.8
″	″	″	″	10	0.1	0.8
Mullin and Park, 1994	N.S.	10	3	5	0.08	0.84
″	″	″	″	10	0.05	0.7
Nelson and Mohn, 1991	Minnesota	190	3	2 (nursery)	0.43	0.52
″	″	″	″	3 (nursery)	0.53	0.63
″	″	″	″	7	0.1	0.35
″	″	″	″	10	0.11	0.34
″	″	″	″	12	0.11	0.36
Boyle, 1986	Ontario	349	19	10	0.12	0.88

N.B. = New Brunswick, N.S. = Nova Scotia

As in the case of population-level studies, family and individual clone rankings are not stable when comparing early growth (seedlings younger than about age 5) to field performance at age 10 or older (Sulzer *et al.,* 1993; Mullin and Park, 1994). The proportion of additive to nonadditive genetic variance (the latter comprised of mainly epistatic rather than dominance variance) for traits such as growth is initially high, but decreases over time (Boyle, 1987; Mullin and Park, 1994). Best correlations between 25-week greenhouse heights and 10-year field heights occur with half-sib families, followed by full-sib families, with clonal rankings showing the weakest relationship (Mullin and Park, 1994). Evidence presented by these authors suggests that culling the very poorest families might be feasible based on early performance, but early individual-level selections would not be reliable.

Numerous other traits of black spruce display high levels of genetic variation. Differences have been described in cone size, seed production, seed size, germination, phenology, seedling biomass, seedling heat tolerance, and morphology (Morgenstern, 1969a, 1969b; Khalil, 1984b; Stoehr and Farmer, 1986; Colombo *et al.,* 1992). Maternal effects are apparent in traits correlated with seed weight and in germination and early growth (Mullin, 1985). Seedling variability in response to soil fertility and nitrogen fertilisation occurs (Maliondo and Krause, 1985; Mullin, 1985). The period of time that a ramet of black spruce is able to produce rootable cuttings is clone-specific, but until recently not successful beyond 5 to 6 years (Cheliak and Rogers, 1990). Rooting success of 10-year-

old ramets similar to that of seedlings of the same age has recently been obtained in Québec (Michel Rioux, Ministère des Ressources Naturelles du Québec, pers. comm. 2005).

Wood characteristics of this species are under strong genetic control, including fibre dimensions (length, diameter, and thickness), wood density (of both juvenile and mature trees), and solubility (Khalil, 1985). The mode of inheritance for specific gravity appears to be mainly additive (Boyle *et al.,* 1988). Wood density is negatively correlated with height, diameter, and stem volume (Zhang and Morgenstern, 1995), although a few families do not exhibit this trend (Zhang *et al.,* 1996). The negative relationship between growth and wood density appears to be weaker in more favourable environments (Zhang *et al.,* 1996) and it also appears to weaken with age (Koubaa *et al.,* 2000). Villeneuve *et al.* (1987) estimated that wood density classification of families could be carried out between age 12 and 15.

Northern sources appear to have higher net photosynthesis rates in early spring, and earlier decline in net photosynthetic rates during autumn, than southern sources (Johnsen *et al.,* 1996). Leaf carbon isotope discrimination has a strong negative genetic correlation with growth, displays a lack of inbreeding depression, and appears to be controlled mainly by additive gene action (Johnsen *et al.,* 1999). However, no genetic differences in foliar nitrogen concentration were observed (Johnsen *et al.,* 1999), and different genotypes seem to have a similar response to elevated atmospheric carbon dioxide (Johnsen and Major, 1998). Faster-growing sources showed evidence for an ability to tolerate dehydration, being capable of sustaining growth under drought conditions, compared to slower-growing sources that accumulated more abscisic acid and closed their stomata more rapidly under drought stress (Tan and Blake, 1993, 1997).

Isozyme variation in black spruce has been well documented (Boyle and Morgenstern, 1985; Knowles, 1985; Pitel *et al.,* 1987). Somatic embryogenesis research has revealed that family variation occurs in the proportion of genotypes that give rise to phenotypically normal mature somatic embryos (Cheliak and Klimaszewska, 1991). A number of different variant phenotypes resulting from somaclonal variation have been identified over 5 years of testing somatic embryo-derived plants (*e.g.* morphological abnormalities including dwarfism, plagiotropism, and bushiness, and needle abnormalities including variegation and fasciation), with clonal trends noted in variant type (Tremblay *et al.,* 1999). STS markers have revealed polymorphisms (Perry and Bousquet, 1998), but at low levels compared to microsatellite (SSR) markers (Hodgetts *et al.,* 2001).

5.3.3. Resistance to pests

In comparison to associated species, black spruce is relatively pest resistant. Black spruce is less susceptible to spruce budworm (*Choristoneura fumiferana* [Clem.]) than white spruce and balsam fir, possibly due to its late bud flush (Blais, 1957), but is also less susceptible to spruce budworm than red spruce, another species exhibiting a late bud flush (Manley and Fowler, 1969; Osawa, 1989). Whereas spruce budworm feeds on both foliage and cones of white spruce, when on black spruce, it prefers the cones (Prévost, 1990). Manley and Fowler (1969) speculated that resistance to spruce budworm is polygenic, as segregation was not apparent in red × black spruce hybrids.

White pine weevil (*Pissodes strobi* [Peck]) damage is positively correlated to height (Bihun and Carter, 1983). Black spruce was slower-growing and more resistant to weevil damage than the faster-growing Sitka spruce and Norway spruce when tested together in the Pacific Northwest for 26 years (Mitchell *et al.,* 1974, 1990). High variation in frequency of weevil attack occurred in a black spruce provenance test in Maine, where western sources showed less damage than Great Lakes and Maritimes sources (Bihun and Carter, 1983). No seed orchard clonal differences in resistance were observed for spruce cone maggot, spruce seed moth, or *Lepidoptera* spp. during a good cone crop year when overall damage rates were low (Turgeon, 1990).

Slower-growing northern black spruce provenances grown at the Petawawa Forest Experiment Station were more prone to infection by *Armillaria mellea* shoestring root rot than southern populations (Morgenstern, 1975b). Possible causes may have been root growth that was out of phase with the test environment.

6. Tree growth and phenology

Growth rates of black spruce across its geographic range are very diverse. Regional differences are related to climate, while within a region, soil moisture and nutrient levels are influential (Vincent, 1965). Trees in uneven-aged stands tend to grow more slowly than those in even-aged stands. In fire-origin mixed stands of northeastern Ontario, black spruce takes an average of 18 years to reach breast height, compared to 7 or 8 years for trembling aspen (*Populus tremuloides* Michx.), white birch (*Betula papyrifera* Marsh.), and jack pine (*Pinus banksiana* Lamb.) (Vasiliauskas and Chen, 2002). The growth rate of black spruce increases until about age 7 to 9, then decreases (Yang and Hazenberg, 1994). Stands at higher density exhibit slower growth patterns. The transition from juvenile to mature wood occurs at approximately age 15, regardless of stand density (Yang and Hazenberg, 1994).

While generally slow-growing, on upland sites, black spruce growth rates may approach those of white spruce. Best growth is achieved throughout a broad region from southern Québec to Saskatchewan. On good unmanaged sites, trees may reach 12 to 20 m in height and 23 cm dbh, but on poor sites, trees may only attain 8 to 12 m in height and 13 cm dbh (Viereck and Johnston, 1990). Heights of 27 m with dbh of 46 cm have been observed in the Ontario clay belt in stands over 140 years old (Heinselman, 1957).

By contrast, on extreme sites such as the northern taiga region or poor muskegs, growth may be minimal; trees may be shrubby, only 3 to 6 m tall with dbh of 3 to 5 cm, even after 100 years (Heinselman, 1957; Vincent, 1965). At the northern limits of its range, black spruce exhibits a range of growth forms that develop in response to gradients in temperature, snow depth, and wind associated with the winter environment (Lavoie and Payette, 1992; Boivin and Bégin, 1997). The forms range from regular symmetric trees to prostrated, matted growth less than 30 cm in height, with various intermediate forms of shrub, reiterated branch, and bare stem complexes (Payette, 1974 as cited in Lavoie and Payette, 1992).

Black spruce is longlived for a boreal species, commonly reaching 200 to 250 years. Generally, this species survives longer in swampy sites than on upland sites, as on the latter butt rot may occur between ages 70 to 100, causing trees to be subject to windthrow (Heinselman, 1957). Deterioration on lowland sites generally occurs after 130 to 180 years. It is common to obtain total volumes on good sites of 196 m^3 / ha by age 80 to 100, with one report of a volume of 492 m^3 / ha at age 100 (Vincent, 1965).

Phenology varies widely across the range of black spruce. Mitotic activity and swelling of vegetative buds begin prior to budburst; the length of this period is dependent upon spring weather conditions. Budburst takes place when soils are not much warmer than freezing, and occurs on average about 7 to 10 days after white spruce has flushed (Vincent, 1965). Budswell is noticable between mid-April and mid-May in the Lake States (Heinselman, 1957). In this region, budburst occurs between the beginning and middle of June, and budset has occurred by August 1 to 10 (Vincent, 1965). In Maine, budburst has been observed between May 17 and June 17, with budset between mid- to late August (Heinselman, 1957). In northern Ontario, budburst occurs between early and mid-June; in the Alberta foothills, vegetative growth typically commences around June 1, and ends around August 2 (Vincent, 1965).

Radial growth in the Lake States takes place between about mid-May to August (Heinselman, 1957). In northern Ontario, radial growth occurs between June 1 and September 30 (Heinselman,

1957). At Chalk River, Ontario, radial growth begins between May 10 and May 25, ending between July 30 to August 10, a period averaging 78 days (Vincent, 1965).

7. Ecology

7.1. Habitat

7.1.1. Climate

Black spruce occupies cold boreal habitats, with moisture regimes ranging from humid to dry subhumid. Viereck and Johnston (1990) list the following temperature and precipitation ranges for the species: mean annual temperature ranging from 7°C to -11°C near the treeline; average January temperatures between -30°C in the northwest to -6°C in the southeast; extreme recorded low of -62°C; extreme recorded high of 41°C; mean annual precipitation decreasing from east to west, from 1,520 mm in the Maritimes to 150 mm in Alaska; snowfall from 500 cm in eastern Canada to 100 cm in western Canada and Alaska; and mean snowdepth of 50–75 cm, but over 100 cm in parts of Québec and Labrador. Snow may last until late May or early June in some areas.

The frost-free period ranges from 130–140 days in the southeast part of the range to less than 60 days in the north, and permafrost is found north of 62°N latitude (Heinselman, 1957). The longest daylength at summer solstice varies from 24 hours above the Arctic Circle to 16 hours near the southern limit (Viereck and Johnston, 1990). Growing degree-day estimates (5°C base) vary from 100 to 2,700, with 80% of the species found between 500 and 1,500 growing degree-days (Thompson *et al.,* 1999).

7.2.2. Soils and site type

Black spruce is most commonly found on wet organic soils, but is also found on other soil types including deep humus, clays, loams, sands, coarse till, boulder pavements, and shallow soils over bedrock (Viereck and Johnston, 1990). Black spruce can tolerate conditions ranging from very wet to dry, and can withstand flooding up to 48 days (Ahlgren and Hansen, 1957). In the southern portion of the range, black spruce is found mainly in peatlands with peat or organic layers greater than 30 cm thick, although it also occurs on upland sites and on transitional sites between peatland and upland (Johnston and Smith, 1983). The most productive sites in the U.S. tend to be transitional sites with shallow organic to wet mineral soils. In New England, black spruce is most often associated with the Adirondack-New England highlands. In the portion of the range adjacent to the Great Lakes, this species commonly inhabits Histosol peat bogs, swamps formed in old glacial beds, muck-filled seepages, stream courses, and lake-swamp-moraine plains; on these sites, peat deposits may be from 0.5 to 6 m deep (Rudolf, 1965; Johnston and Smith, 1983; Viereck and Johnston, 1990). On peatlands of the Great Lakes region, best productivity occurs on dark brown to black peat with high decomposed woody matter containing water-borne nutrients from adjacent mineral soils, while lowest productivity occurs on thick deposits of partially decayed sphagnum (Johnston and Smith, 1983; Viereck and Johnston, 1990).

In the absence of disturbance such as fire, black spruce is usually confined to wet or occasionally very dry sites, but with disturbance, this species is able to inhabit rich upland environments (Fowler and Park, 1982). Productivity is higher on the upland sites than the lowlands. In central and eastern Canada, upland sites include podzolic Spodosols and, on gentle slopes underlain by glacial till-origin clays, gley Inceptisols (Viereck and Johnston, 1990). These clay soils are derived from calcarious material and are thus neutral or slightly alkaline. The most productive soils are on better drained sites such as sandy glacial deposits, river terraces, and Entosol outwash plains in association with hardwoods (Viereck and Johnston, 1990). Black spruce on glacial tills of predominantly sandy loams

and loamy sands has been found to have higher productivity than those on very-well drained fluvioglacial or alluvial deposits (Hamel *et al.,* 2004). Total net primary productivity of a closed-canopy site having a feathermoss ground cover over moderately drained soils was significantly greater than that of an open-canopy site of similar age having *Sphagnum* ground cover over poorly drained soils (O'Connell *et al.,* 2003).

At the northern extent of the range, the shallow, poorly developed mineral soils are underlaid by permafrost. Due to its shallow rooting, black spruce is the species best adapted to permafrost conditions (Viereck and Johnston, 1990). Black spruce sites of the taiga in the interior of Alaska are the most fire-prone, most nutrient-limited, and least productive sites of that region (Van Cleve *et al.,* 1982). Such sites are low in nitrogen, have high lignin content, and are very acidic. Wildfire in permafrost regions temporarily increases the thaw depth (Viereck and Johnston, 1990), and for 10 to 20 years following fire, soils will be warmer, and these sites will be more productive (Van Cleve *et al.,* 1982).

7.2. Synecology and associated species

Black spruce is often found in pure stands on organic soils, and is commonly found in mixed stands on mineral soils. At the northern treeline, black spruce may be in pure stands or interspersed with white birch, trembling aspen, white spruce, tamarack (*Larix laricina* [Du Roi] K. Koch), or balsam poplar (*Populus balsamifera* L.). Common associates across most of the species range include white spruce, trembling aspen, balsam fir (*Abies balsamea* [L.] Mill.), white birch, and tamarack (Viereck and Johnston, 1990). Eastern white cedar (*Thuja occidentalis* L.) and red spruce may also be stand components in the southeastern portion of the range. Infrequently in the east, black spruce may be a minor component in moist mixed stands containing black ash (*Fraxinus nigra* Marsh.), American elm (*Ulmus americana* L.), and red maple (*Acer rubrum* L.), or in the understory below red pine (*Pinus resinosa* Ait.) and eastern white pine (*Pinus strobus* L.) (Heinselman, 1957). On dry sands and gravels, black spruce may succeed jack pine, while in the west, black spruce may succeed lodgepole pine (*Pinus contorta* Dougl. ex Loud.) on moist loamy tills and well-drained uplands (Vincent, 1965). In the northwest at higher elevations, black spruce occasionally grows with subalpine fir (*Abies lasiocarpa* [Hook.] Nutt.) (Heinselman, 1957).

On better sites in the eastern portion of the range, shrubs found in association with black spruce include mountain maple (*Acer spicatum*), beaked hazel (*Corylus cornuta*), speckled alder (*Alnus rugosa*), red-osier dogwood (*Cornus stolonifera*), and red raspberry (*Rubus idaeus*) (Viereck and Johnston, 1990). On eastern sites of lower productivity, shrub associates include dwarf birches (*Betula pumila* and *B. glandulosa*), bog-rosemary (*Andromeda glaucophylla*), Kalmia (*Kalmia angustifolia* and *K. polifolia*), Labrador-tea (*Ledum groenlandicum*), and leatherleaf (*Chamaedaphne calyculata*) (Viereck and Johnston, 1990). In the west, common shrubs include willows (*Salix arbusculoides*, *S.glauca*, and *S. bebbiana*), prickly rose (*Rosa acicularis*), green alder (*Alnus crispa*), and blueberry and cranberry (*Vaccinium* spp.).

Feathermosses and sphagnum mosses provide nearly continuous ground cover in many black spruce stands, but are typically replaced by lichens in open areas in the north (Viereck and Johnston, 1990). Herbaceous species common to much of the black spruce range include panicle bluebell (*Mertensia paniculata*), fireweed (*Epilobium angustifolium*), one-sided pyrola (*Pyrola secunda*), twinflower (*Linnaea borealis*), bunchberry (*Cornus canadensis*), wild sarsaparilla (*Aralia nudicaulis*), false lily-of-the-valley (*Maianthemum canadense*), starflower (*Trientalis borealis*), bluejoint reedgrass (*Calamagrostis canadensis*), and sheathed cottonsedge (*Eriophorum vaginatum*) (Viereck and Johnston, 1990).

7.3. Competition, succession, and stand structure

Black spruce, although shade-tolerant, is unable to compete with faster-growing associates including balsam fir, jack pine, tamarack, white birch, and trembling aspen unless conditions are unsuited to these species. Where they occur together, black spruce may have more rapid juvenile growth than white spruce (Heinselman, 1957). However, black spruce generally requires site disturbance to be able to become established on rich upland sites, and without further disturbance, it is unlikely to regenerate on these sites, being succeeded by balsam fir, white spruce, and white birch. At the edge of bogs in association with advancing sedge mats, black spruce is one of the first pioneer species, preceded by tamarack (Heinselman, 1957).

Black spruce is well adapted to post-fire establishment, chiefly through its abundant production of semi-serotinous cones, which maintain an aerial seed bank that is readily released in the months and years immediately following fire (Viereck, 1983). Seedling establishment may begin in the year following fire, though reported recruitment rates vary. St-Pierre *et al*. (1992), Lavoie and Sirois (1998) and Greene *et al.* (2004) found that most seedlings became established within 3 years of fire. Lieffers (1986), Sirois and Payette (1989), and Landhäusser and Wein (1993) observed peak recruitment 5 to 8 years following fire. Studying post-fire recruitment in the Yukon and Alaska, Johnstone *et al.* (2004) found that on a mixedwood site, black and white spruce did not reach 50% of net establishment until 5 to 10 years after fire; on a dominantly black spruce site, 50% of net establishment occurred within 3 years of fire, with little change after 10 years. In Québec, black spruce mean fecundity (measured as recruits per square metre of parent basal area) increased with increasing fire severity, a result attributed to a reduction in the rodent population and granivory rate (Greene *et al.,* 2004). Though black spruce can reproduce vegetatively by layering, this does not appear to be a significant mechanism of early post-fire re-establishment.

Pattern, intensity and frequency of forest fire influence the nature of regeneration and successional pathways of black spruce types. Seedbed quality and seed germination and survival may be affected by fire intensity (Carleton, 1982; Zasada *et al*., 1983; Zasada *et al.,* 1992 as cited in Berger and Gilmore, 2003; Sirois, 1993), causing long-term impacts on subsequent stand development (Arseneault, 2001). However, Berger and Gilmore (2003) observed no significant difference in the number of spruce germinants, or their winter survival, among three levels of burn intensity following prescribed burns in Alberta. Forest fires of sufficient intensity serve to remove competition, enhance the seedbed, and open cones causing seed dispersal, which combine to promote the establishment of dense even-aged black spruce stands (Johnson, 1956; Black and Bliss, 1978). This is often the case throughout much of the range of black spruce. In some cases, white birch and trembling aspen may initially invade black spruce sites following fire, to be subsequently replaced by spruce (see Viereck, 1983). In maritime Labrador, a relatively long fire cycle allows for the accumulation of a thick organic layer, which results in slow re-establishment of black spruce and an uneven-aged structure (Foster, 1985). In areas supporting both black spruce and jack pine, a short fire interval may favour the more precocious jack pine (Lavoie and Sirois, 1998; Larocque *et al*., 2000; Parisien and Sirois, 2003). Simulation work by Goff and Sirois (2004) showed that a fire interval less than 60 years may lead to the local extinction of black spruce on well-drained but not poorly drained sites in Québec. The more shade-tolerant black spruce, however, can establish in the subcanopy of jack pine dominant stands (Carleton, 1982). In the Abitibi Region of Québec, Harper *et al*. (2002) observed changes in stand-level canopy dominance during the 100 years following fire, with stands initially dominated by deciduous species and jack pine shifting to black spruce composition.

In the absence of fire, black spruce may be succeeded by more shade-tolerant balsam fir and white cedar (Hatcher, 1963; Viereck, 1983), and white spruce and white birch (LeBarron, 1948). In Québec, Cogbill (1985) found that stands over 200 years of age had poor growth and limited reproduction; layers only partially filled gaps created by mortality. Foster (1985) suggested that black spruce layers

and balsam fir seedlings effectively replaced senescent trees of a 230-year-old cohort, maintaining a closed, multi-aged canopy. In the absence of disturbance, uneven-aged, self-perpetuating stands appear to develop on nutrient-poor peatland sites in Ontario, as mortality within the initial cohort is replaced by recruitment from layers (Groot and Horton, 1994). In Québec's North Shore region, an area having a long fire cycle, Pham *et al.* (2004) found that older, black spruce dominated stands were self-replacing; gaps caused by successive events of individual tree mortality were regenerated, largely through layering. The importance of gap dynamics in the development of old growth black spruce stands was also noted by Harper *et al*. (2003) in the Clay Belt region of Ontario and Québec.

7.4. Species interactions and dynamics

Due to its shallow rooting habit and vulnerability to root rot, black spruce may be susceptible to wind damage through stem breakage and uprooting, particularly in older stands and along cut edges (LeBarron, 1948; Robinson, 1974; Whitney and Fleming, 1995). Heavy snow load occasionally causes crown breakage (Van Cleve and Zasada, 1970). Although very cold-hardy and at low risk of spring frost damage due to its late flushing habit, black spruce experiences occasional frost damage. Vincent (1965) described black spruce vulnerability to drought, while excessive flooding, particularly in peat bogs, also occurs (Heinselman, 1957). Both crown fires and ground fires can cause black spruce mortality (Heinselman, 1957). Peat bogs are less prone to fires except when conditions are very dry (Johnston, 1977).

Eastern dwarf mistletoe (*Arceuthobium pusillum* Peck.) is a serious disease of black spruce in the Lake States and eastern Canada, less so in the western part of its range (Heinselman, 1957). Affecting both small seedlings and larger trees (LeBarron, 1948), the disease stunts growth, causes witches'-broom, and often kills trees. In northern Minnesota, Baker and Knowles (2004) suggested that all residual black spruce should be eradicated after harvesting infested stands, to prevent unacceptable losses in the regenerating stands.

Black spruce is susceptible to a number of decay fungi that result in stem and root rots. These include Armillaria root rot (*Armillaria* spp), tomentosus root rot (*Inonotus tomentosus* [Fr.] Teng), velvet top fungus (*Phaeolus schweinitzii* [Fr.:Fr.] Pat.), red ring rot (*Phellinus pini* [Brot.:Fr.] A.Ames), *Coniophora puteana* (Schumach.:Fr., P.Karst.) and *Climacocystis borealis* ([Fr.:Fr.] Kotl. & Pouzar) (Johnston and Smith, 1983; Myren and Cameron, 1995). Rot may cause direct mortality, reduced height and diameter growth, and greater susceptibility to windfall (*e.g*. Livingston, 1990; Whitney, 1994; Whitney and Fleming, 1995). Losses can be significant: Whitney (1989) estimated that 23% of the total merchantable volume was lost due to rot in sampled black spruce stands in Ontario.

Spruce budworm is one of the most important insect pests of black spruce (Johnston and Smith, 1983). The budworm is indigenous to eastern North America, and outbreaks occur in spruce-fir stands in the central and southern Boreal, the northern Great Lakes-St. Lawrence, and Acadian forest regions (Blais, 1983). Black spruce is less susceptible to budworm than balsam fir or white spruce, a response attributed to the later flushing of black spruce relative to the spring emergence of the budworm (Blais, 1957, Nealis and Régnière, 2004). In a boreal mixedwood stand in Ontario, mortality due to budworm was relatively low for black spruce, whereas most of the codominant and intermediate balsam fir had been killed (90% basal area reduction) and white spruce been reduced by half (50% basal area reduction) (Nealis and Régnière, 2004). Nevertheless, where it occurs in mixed stands with these species, black spruce may also be affected. Studying a budworm outbreak in Québec, Bouchard *et al.* (2005) found mortality rates for black spruce of 78.6% in mixed boreal stands and 56.6% in balsam fir dominated stands. In mixed stands, the mortality of balsam fir may leave black spruce susceptible to windfall (Heinselman, 1957). Pure black spruce stands, though less vulnerable, may also be attacked (Raske and Sutton, 1986). Spruce budworm defoliations for several successive years can cause growth reductions and mortality (Elliot, 1960; Krause and Morin, 1999), and may leave black spruce

susceptible to damage by secondary agents, such as bark beetle (*Polygraphus rufipennis* [Kirby]) and root rot (*Armillaria* spp.) (Raske and Sutton, 1986). The budworm may cause significant damage to flowers and cone crops (Schooley, 1980; Prévost *et al.,* 1988).

The yellow-headed spruce sawfly (*Pikonema alaskensis* [Roh.]) may severely defoliate young, open-grown black spruce (Martineau, 1984; Syme, 1995); repeated defoliations may result in loss of vigour and mortality (Hopkin *et al.,* 1994b). The green-headed spruce sawfly (*Pikonema dimmockii*) occasionally attacks black spruce (Martineau, 1984; Viereck and Johnston, 1990). In eastern Canada, the European sawfly (*Diprion hercyniae* Htg.) may cause heavy defoliation, growth reduction and mortality (MacAloney, 1936; Reeks and Barter, 1951).

White pine weevil may feed on black spruce (Belyea and Sullivan, 1956), resulting in girdling and death of the top portion of the main stem (Syme, 1995). Hopkin *et al*. (1994a) found that only a small percentage of trees in Ontario plantations were affected, chiefly those 2 to 6 m in height.

The spruce coneworm (*Dioryctria reniculelloides* Mut. and Mun.) feeds on foliage and cones of black spruce (West, 1986; Syme, 1995). It is often found in association with spruce budworm, and may cause relatively more (Spies and Dimond, 1985) or less damage (Ives and Wong, 1988; Prévost *et al.,* 1988). The black spruce cone maggot (*Strobilomyia appalachensis* Michelsen) and the white spruce cone maggot (*Strobilomyia neanthracina* Michelsen) feed internally on seeds within cones and may significantly reduce seed production without adversely affecting vegetative growth (Syme, 1995; Wanner *et al.,* 1995).

Red squirrels (*Tamiasciurus hudsonicus* [Erxleben]) frequently harvest cones of coniferous species, including those of black spruce (West and de Groot, 1990). In Ontario, cone losses of 18 to 28% have been reported (Prévost *et al.,* 1988; Wanner *et al*., 1995). In Newfoundland, West (1989) observed cone losses of 64 to 96% in small crop years, but only 1% in a good crop year. Seed and seedling predation by small rodents (e.g., deer mouse (*Peromyscus maniculatus* Wagner), red-backed vole (*Clethrionomys gapperi* Vigor) and heather vole (*Phenacomys intermedius* Merriam)) can adversely affect post-fire seedling establishment of black spruce (Côté *et al.,* 2003). Côté *et al.* (2005) found that seed predation by invertebrates ranged from 9% to 19%, varying with habitat type; juvenile seedling predation ranged from 2% to 12%. A variety of bird species utilise black spruce for food and cover including spruce grouse, ruby-crowned kinglet, magnolia warbler, Cape May warbler, ovenbird, pine grosbeak, pine siskin, and crossbill (Viereck and Johnson, 1990). Moose (*Alces alces americana*) and white-tailed deer (*Odocoileus virginianus*) may browse on black spruce, but it is not a preferred food source (Heinselman, 1957). Snowshoe hares (*Lepus americanus*) may cause significant damage to young spruce by debarking stems and removing leaders and branches (Heinselman, 1957). Numerous other mammals are associated with black spruce habitat for forage and/or cover, including mouse, vole, chipmunk, muskrat, shrew, mink, porcupine, raccoon, skunk, beaver, and black bear (Viereck and Johnston, 1990; Parish and Thomson, 1994).

In older black spruce stands, small-scale disturbances due to windthrow may be common. Harper *et al.* (2002) found that 16% of the area occupied by 100- to 250-year-old black spruce was affected by windthrow; fine, coarse and thin soil site types were more adversely affected than organic sites.

8. Reforestation practices

8.1. Provenance transfer

Black spruce was reportedly first introduced into Europe in the 18th century (Sargent, 1898 as cited in Morgenstern and Farrar, 1964). Provenance testing of this species has been carried out in Germany, Norway, and several other European countries (Morgenstern, 1969a, 1996; Braekke, 1990).

Provenances performing best in Newfoundland were from Newfoundland, Prince Edward Island, and Nova Scotia (Hall, 1986), although Morgenstern and Mullin (1990) recommended that fast-growing sources from New Brunswick, Ontario, and Québec could also be transferred to Newfoundland. Khalil (1984a) suggested that intensive family selection would be most appropriate for Newfoundland, while Hall (1986) recommended using well-performing sources from within each forest region.

In the Maritimes, Maine, and Minnesota, best-growing sources were from southern Great Lakes sources (Fowler and Park, 1982; Bihun and Carter, 1983; Merrill *et al.,* 1984). In northern and central Maine, source transfer 1 to 2° northward was suggested for best growth, in conjunction with Prince Edward Island and New Brunswick sources, while in southern New Brunswick, local sources plus those from Prince Edward Island were recommended (Fowler and Park, 1982; Park and Fowler, 1988). These authors also advised using local sources plus New Brunswick sources in Prince Edward Island. They recommended use of 3 overlapping breeding zones for the Maritimes. Black spruce populations in Nova Scotia may actually be mainly composed of red × black spruce hybrids; local sources exhibited comparatively poor growth, prompting recommendations of Prince Edward Island and central / southern New Brunswick sources (Fowler and Park, 1982; Park and Fowler, 1988). Poor growth of Cape Breton, Nova Scotia provenances was observed at all Maritimes sites. These sources are from geographically isolated populations that are phenologically out of phase with the rest of the region, and probably inbred (Fowler and Park, 1982). In that region, local sources plus additional sources from Newfoundland and northern New Brunswick have been recommended.

Sources from the southwest portion of the black spruce range grew best in Québec, while poorest growth was from sources from the northeast portion of the range (Beaulieu *et al.,* 1989). Most (50–75%) of the observed variation was within provenances. The authors suggested 5 breeding zones for Québec, and recommended incorporation of well-performing provenances from outside of the province into their black spruce programs. In Ontario, best performance was observed in sources from central and western Ontario and Québec (Boyle, 1985). Local sources were recommended for the Ottawa River vicinity (Morgenstern and Mullin, 1990). Significant genotype by environmental interactions occurred in Ontario provenance tests (Boyle, 1986). Low-intensity, rapid selection was recommended for black spruce in Ontario. In the prairies, best growth was from southern provenances (Morgenstern and Mullin, 1990). Early provenance trial results in Manitoba indicated that seed source selection may be more important on poor, nutrient-deficient sites compared to better sites, where choice of seed source appeared less critical (Segaran *et al.,* 1978). Based on these early results, southern provenances grew best when transferred north (between 2 and 5° latitude), and outperformed local sources, whereas northern provenances grew less when transferred south (Segaran, 1979).

8.2. Breeding programs

Black spruce breeding programs have been established in most jurisdictions where the species occurs naturally, and are of greatest importance in the eastern portion of the range. Selection is primarily based upon stem growth and wood quality traits, as stem and crown form are fairly uniform in this species (Morgenstern and Park, 1991). New Brunswick has one of the most advanced programs, where second generation seed orchards were initiated in 1989 and are now producing enough seed for the province's reforestation needs. Nova Scotia and Prince Edward Island have also established second generation seed orchards, but are not yet producing significant quantities of seed. Ontario and Québec are in the process of establishing second generation seed orchards. Alberta, Manitoba, and Newfoundland all have first generation seed orchards. In the U.S., first generation seedling seed orchards have been established in Maine, Vermont, and Minnesota (Carter *et al.,* 1988; Nelson and Mohn, 1991). Updates of the Canadian programs are regularly presented in the biannual Canadian Tree Improvement Association proceedings members' reports.

The majority of first-generation programs are based on open-pollinated family selection, with seed orchard roguing typically carried out after progeny tests are measured at around age 10. Second-generation strategies are more diverse, and often include stratification of the breeding population. For instance, New Brunswick is using a complimentary mating design with polycrossing for estimation of breeding values, and sublining with assortative mating for the breeding population (Park *et al.,* 1993). One elite subline consisting of the best material will use a disconnected diallel, while the regular sublines use a weighted assortative mating scheme, with the best material used in more crosses (Park *et al.,* 1998). Ontario has adopted a nucleus breeding system, with the breeding population substructured into elite and infusion populations (Cherry and Joyce, 1998). Amalgamation of black spruce seed zones in northwestern Ontario has recently been carried out using GIS-based Focal Point Seed Zone methodology (Parker, 1992). In Québec, a GIS-based tool is used to guide seed source transfer. This tool was developed with Campbell's relative risk concept, land district maps, and mathematical models to relate variation in adaptive traits to geoclimatic characteristics of seed source (Beaulieu *et al.,* 2004).

The black spruce program in Québec is experimenting with both containerised seed orchards and field-based miniature orchards, both of which are intensively managed (Mercier and Périnet, 1998). These alternative orchard types are both easily manipulated, with the goal of early, abundant seed production. This province is also examining methods to improve pollination efficiency and to partially shelter orchards by using polyhouse tunnel covers or strategic windbreaks (Mercier and Périnet, 1998). Deployment of stock as mosaics of family blocks is being compared to deployment of family mixes in New Brunswick (Adams and Tosh, 1998).

Unequal clonal contributions to seed orchard seed crops have been observed, necessitating intervention to achieve better balance such as adjusting cone harvesting by clone, partial roguing, preferential flower induction, and supplemental mass pollination (Adams and Kunze, 1996). Isozyme studies of black spruce have been proposed as useful in identification of pollen contamination, checking for errors in seed handling, controlled crossing, and clonal propagation (Pitel *et al.,* 1987), and in making inferences regarding nonrandom mating in seed orchards (Knowles, 1985).

8.3. Reproductive propagation

8.3.1. Flower induction

Gibberellin $GA_{4/7}$ has been successfully used to promote female flowering, as a foliar spray (Greenwood *et al.,* 1988) and also *via* stem injection (Mercier and Périnet, 1998). $GA_{4/7}$ is particularly effective in heavy cone crop years (Brockerhoff and Ho, 1997). However, male strobilus production has been very inconsistent in response to $GA_{4/7}$ application (Greenwood *et al.,* 1988). Stem injections are easier to apply than foliar sprays, particularly in taller trees such as those in seed orchards, and are also not affected by precipitation or aerial drift (Brockerhoff and Ho, 1997). Québec and New Brunswick both use stem injections of $GA_{4/7}$ in their breeding programs (Simpson, 1997; Mercier and Périnet, 1998).

Québec researchers have also investigated using red light flashes (interrupting the nightly dark period) during spring as a way to induce flowering without undue stress (Mercier and Périnet, 1998). New Brunswick has refined crown management techniques and used nitrogen fertilisation for promoting flowering, and has used accelerated growth cycles of grafted seed orchard stock prior to outplanting to promote early flowering (Simpson, 1997; Simpson and Tosh, 1997; Adams and Tosh, 1998). Other operational techniques to stimulate flowering include root pruning and water stress.

8.3.2. Vegetative propagation

Black spruce may be vegetatively propagated through rooted cuttings, not unexpected in light of the ability of this species to layer naturally. Black spruce clonal forestry programs have been initiated, although early challenges included inability to maintain juvenility, plagiotropism, poor root development, and high production costs (Cheliak and Rogers, 1990; deWitt, 1990a, 1990b; Kleinschmidt, 1992). The New Brunswick Tree Improvement Council has refined rooted cutting techniques for both dormant (hard) and semi-lignified (soft) cuttings; both are used in reforestation with improved stock (Adams and Tosh, 1998). Black spruce grafts readily, and grafts are utilised in clonal seed orchards and clone banks, although grafting success may be influenced by the very small scions sometimes obtained from mature black spruce (Morgenstern and Park, 1991). Québec is using rooted cuttings instead of grafts for establishing second generation seed orchards (Tousignant *et al.,* 1995; Mercier and Périnet, 1998) and also produces over 2 million rooted cuttings annually for the reforestation program.

Somatic embryogenesis may be used in clonal breeding programs to capture nonadditive gain. This procedure may also be useful in preserving genotypes, which would circumvent the difficulty in attempting to retard aging in black spruce clone banks until field testing has been carried out and rooted cuttings of selected genotypes can be struck. The first report of somatic embryogenesis in black spruce was by Hakman and Fowke (1987), who established embryonic callus using immature zygotic embryos. Since then, somatic embryogenesis culturing techniques of both zygotic and mature embryos have been refined for this species. Tautorus *et al.* (1990) successfully derived embryonic tissue from mature embryos of seed that had been stored for 13 years. Attree *et al.* (1990a) regenerated plantlets from 12-day-old seedlings, and plantlets were successfully transferred to soil (Attree *et al.,* 1990b). Cryopreservation of embryonic black spruce cultures is now possible (Klimaszewska *et al.,* 1992). Encapsulation procedures that allow handling of fragile embryos, and offer the potential for automated planting at operational scales, have been developed (Lulsdorf *et al.,* 1993). Somatic embryogenesis has now been widely implemented in numerous black spruce genotypes (Adams *et al.,* 1994).

Gene transformation has been demonstrated in black spruce using *Agrobacterium*-mediated, microprojectile particle bombardment, and electroporation techniques (Klimaszewska *et al.,* 2003; Tang and Newton, 2003).

8.4. Stock deployment

Artificial regeneration of black spruce may take the form of planting seedlings or rooted cuttings, or aerial seeding. While bareroot seedling production was common in the past, currently most planting material is container stock grown in greenhouses. Emblings resulting from somatic embryogenesis are now available, and may be integrated into planting programs in the near future once operational techniques have been developed.

Planting programs are well-developed in eastern Canada and the northeastern U.S., and black spruce is the most widely planted species in eastern Canada. According to the National Forestry Database Program (Canadian Council of Forest Ministers, 2002), over 307 million spruce seedlings were planted across Canada in 2000, accounting for 50.1% of all planting stock. In Québec, over 80% of the area harvested annually is now being naturally regenerated. In British Columbia, little to none of the spruce planted is black spruce; if this province is eliminated from the total, about 242 million spruce seedlings were planted in the remainder of Canada, the majority of which was black spruce. Species breakdowns were not available for amount of land aerial-seeded and number of rooted cuttings planted.

8.5. Conservation of genetic resources

Black spruce is widespread and abundant, and easily propagated with few barriers to gene flow. While studies have indicated little difference in diversity measures between natural and plantation stands including those originating from clonal seed orchards (Knowles, 1985), and between logged layer-origin stands and fire regenerated seedling-origin stands (Perry and Bousquet, 2001), genetic diversity of the species may be influenced by both domestication and by reduction of the forest landbase. Populations at the extremes of the range, which may be more isolated with more frequent inbreeding and purging of genes, are perhaps most at risk of loss of diversity. Selection against certain genotypes may occur during cone collection, nursery practices, tree improvement programs, precommercial thinning, and harvesting. Reduction of the gene pool and loss of coadapted gene complexes may result in elimination of genes that would be advantageous for future adaptation.

Concerns regarding depletion of the black spruce gene pool as a result of 50 years of extensive harvesting in northern Ontario had been expressed over 2 decades ago, leading to the development of an early program to preserve the gene pool (Brown, 1979). New Brunswick implemented a reserve-stand policy for black spruce as a method of *in situ* conservation (Fowler, 1986). A recent investigation, based on black spruce allozymes of Manitoba populations, claims that the effects of clearcut harvesting on genetic diversity are not significantly different than those due to forest fires (Rajora and Pluhar, 2004).

In situ conservation may be practiced through maintenance of forest reserves and protected areas, and, in regions where population fragmentation occurs, migration corridors. Reserves that are sufficient to allow for natural evolutionary processes such as mutation, gene flow, mating, and selection to occur are preferable. Control over seed transfer and stand management practices aimed at increasing diversity may provide further conservation measures. Populations most at risk of genetic depletion need to be identified, and steps taken to protect them.

Ex situ conservation may be most useful in preserving material from populations facing the greatest risk of gene loss. *Ex situ* conservation includes storage of seed in seed banks, germplasm by cryopreservation, clone banks, seed orchards, field trials, and even plantations. Most provinces operate their own seed storage facilities. Natural Resources Canada also operates a National Forest Genetic Resources Centre (which includes the National Tree Seed Centre) based at the Atlantic Forestry Centre, with the goal of gene conservation of species native to Canada (Simpson and Daigle, 1998). Samples from across the range of a species are being obtained. The multiple population breeding system is designed to manage populations in tree breeding programs in such a way that genetic diversity is maintained or even increased, despite reduction of the population size through ongoing selection (Ericsson *et al.,* 1993). This system thus offers a viable strategy that would be applicable in black spruce tree breeding programs.

9. Summary

Black spruce, one of the most widespread species in North America, is of great economic importance in eastern Canada and northeastern U.S. due to its high pulpwood quality. This boreal species is unique in its ability to be both a pioneer species on disturbed upland habitat, as well as a late-successional species on lowland sites. Black spruce is very resilient, able to survive under extreme environmental conditions, and can occupy sites where other species cannot survive. This species is late-flushing, and normally not as susceptible to spring frosts as is white spruce. It is less affected by insect or disease pests than many associate species.

Black spruce, with its large contiguous range and extensive gene flow, is a predominantly outcrossing, genetically variable species. It is easily regenerated both vegetatively through layering and by seed. The persistent, semi-serotinous cones can remain on the tree for many years with seed remaining viable. Black spruce is adapted to fire ecosystems, and opportunities presented by disturbances such as fire allow the species to readily regenerate.

Although the range of black spruce is very similar to that of white spruce, the two species rarely hybridise, and evidence points to mating barriers such as asynchronous flowering and bud phenology. Black spruce does hybridise with red spruce, which is found in the eastern portion of the black spruce range. Black spruce and red spruce appear more closely related to each other than to other North American members of *Picea*, and seemingly migrated to this continent in a different manner than the other spruces. However, these two species may have undergone divergent evolution as a result of geographic isolation prior to the last glaciation, with further divergent evolution being halted through hybridisation during northward migration with the glacial retreat.

References

Abrams, M.D., C.A. Copenheaver, B.A. Black and S. van de Gevel. 2001. Dendroecology and climatic impacts for a relict, old-growth, bog forest in the Ridge and Valley Province of central Pennsylvania, U.S.A. *Can. J. Bot.* 79: 58-69.

Adams, G.W., and H.A Kunze. 1996. Clonal variation in cone and seed production in black and white spruce seed orchards and management implications. *For. Chron.* 72: 475-480.

Adams, G.W., and K.J. Tosh. 1998. The status and potential of using controlled parentage in operational reforestation in New Brunswick. *For. Chron.* 74: 190-194.

Adams, G.W., M.G. Doiron, Y.S. Park, J.M. Bonga and P.J. Charest. 1994. Commercialization potential of somatic embryogenesis in black spruce tree improvement. *For. Chron.* 70: 593-598.

Adams, W.T. 1992. Gene dispersal within forest tree populations. *New For.* 6: 217-240.

Ahlgren, C.E., and H.L. Hansen. 1957. Some effects of temporary flooding on coniferous trees. *J. For.* 55: 647-650.

Alden, B. 1987. Taxonomy and geography of the genus *Picea*. Int. Dendrol. Soc. Yearb. 1986: 85-96.

Alden, H.A. 1997. Softwoods of North America. USDA FS For. Prod. Lab. Gen. Tech. Rep. FPL-GTR-102, 151 pp.

Andrews, J.T. 1987. The Late Wisconsin glaciation and deglaciation of the Laurentide Ice Sheet. Pp. 13-37 *in* W.F. Ruddiman and J.E. Wright Jr. (eds.), North America and Adjacent Oceans During the Last Deglaciation. Geogr. Soc. Amer., Boulder, Co., The Geology of North America K-3, 501 pp.

Arseneault, D. 2001. Impact of fire behavior on postfire forest development in a homogeneous boreal landscape. *Can. J. For. Res.* 29: 462-473.

Atkinson, G.T., and V.F. Haavisto. 1992. Managing black spruce stands for perpetual seed supply. For. Can. Great Lakes Forestry Centre Frontline Tech Note No. 14, 4 pp.

Attree, S.M., S. Budimir and L.C. Fowke. 1990a. Somatic embryogenesis and plantlet regeneration from cultured shoots and cotyledons of seedlings from stored seeds of black and white spruce (*Picea mariana* and *Picea glauca*). *Can. J. Bot.* 68: 30-34.

Attree, S.M., T.E. Tautorus, D.I. Dunstan and L.C. Fowke,. 1990b. Somatic embryo maturation, germination, and soil establishment of plants of black and white spruce (*Picea mariana* and *Picea glauca*). *Can. J. Bot.* 68: 2583-2589.

Baker, F.A., and K.R. Knowles. 2004. Case study: 36 years of dwarf mistletoe in a regenerating black spruce stand in northern Minnesota. *North. J. Appl. For.* 21(3):150-153.

Baker, R.G., A.E. Sullivan, G.R. Hallberg and D.G. Horton. 1989. Vegetational changes in western Illinois during the onset of Late Wisconsinian glaciation. *Ecology* 70: 1363-1376.

Barrett, J.W., P. Knowles and W.M. Cheliak. 1987. The mating system in a black spruce clonal orchard. *Can. J. For. Res.* 17: 379-382.

Beaulieu, J., A. Corriveau and G. Daoust. 1989. Phenotypic stability and delineation of black spruce breeding zones in Québec. For. Can. Laurentian For. Centre Info Rep. LAU-X-85E, 39 pp.

Beaulieu, J., M. Peron and J. Bousquet. 2004. Multivariate patterns of adaptive variation and seed source transfer in *Picea mariana. Can. J. For. Res.* 34 (3): 531-545.

Belyea, R.M., and C.R. Sullivan. 1956. The white pine weevil: A review of current knowledge. *For. Chron.* 32: 58-67.

Berger, C.A., and D.W. Gilmore. 2003. Germination and survival of spruce seedlings following fire in northwestern Alberta. *North. J. Appl. For.* 20 (1): 45-47.

Berlyn, G.P., J.L. Royte and A.O. Anoruo. 1990. Cytophotometric differentiation of high elevation spruces: physiological and ecological implications. *Stain Technol.* 65: 1-14.

Bihun, Y.M., and K.K. Carter. 1983. Rangewide provenance study of black spruce in northern Maine. Pp. 1-11 *in* Proc. 28th Northeastern Forest Tree Improvement Conf., Univ. New Hampshire, 1982.

Black, R.A., and L.C. Bliss. 1978. Recovery sequence of *Picea mariana – Vaccinium uliginosum* forests after burning near Inuvik, Northwest Territories, Canada. *Can. J. Bot.* 56: 2020-2030.

Blais, J.R. 1957. Some relationships of the spruce budworm, *Choristoneura fumiferana* (Clem.) to black spruce, *Picea mariana* (mariana) Mill. *For. Chron.* 33: 364-372.

Blais, J.R. 1983. Trends in the frequency, extent, and severity of spruce budworm outbreaks in eastern Canada. *Can. J. For. Res.* 13: 539-547.

Bobola, M.S., R.T. Eckert and A.S. Klein. 1992. Restriction fragment variation in the nuclear ribosomal DNA repeat unit within and between *Picea rubens* and *Picea mariana*. *Can. J. For. Res.* 22: 255-263.

Bobola, M.S., R.T. Eckert, A.S. Klein, K. Stapelfeldt, D.E. Smith and D. Guenette. 1996. Using nuclear and organelle DNA markers to discriminate among *Picea rubens*, *Picea mariana*, and their hybrids. *Can. J. For. Res.* 26: 433-443.

Boivin, S., and Y. Bégin. 1997. Development of a black spruce (*Picea mariana*) shoreline stand in relation to snow level variations at Lake Bienville in northern Quebec. *Can. J. For. Res.* 27: 295-303.

Bouchard, M., D. Kneeshaw and Y. Bergeron. 2005. Mortality and stand renewal patterns following the last spruce budworm outbreak in mixed forests of western Quebec. *For. Eco. Man.* 204: 297-313.

Boyle, T.J.B. 1985. Range wide provenance tests of black spruce in Ontario. Canadian Forest Service Petawawa National Forest Institute Info Rep. PI-X-57, 36 pp.

Boyle, T.J.B. 1986. Ten-year height growth of open-pollinated black spruce families in Ontario. Canadian Forest Service Petawawa National Forest Institute Info Rep. PI-X-61, 24 pp.

Boyle, T.J.B. 1987. A diallel cross in black spruce. *Genome* 29: 180-186.

Boyle, T.J.B., and E.K. Morgenstern. 1984. The population structure of black spruce in central New Brunswick. Pp 142-156 *in* E.M. Demeritt (ed.), Proc. Northeastern Forest Tree Improv. Conf. Div. For., W. Va. Univ., Morgantown.

Boyle, T.J.B., and E.K. Morgenstern. 1985. Inheritance and linkage relationships of isozymes of black spruce in New Brunswick. *Can. J. For. Res.* 15: 992-996.

Boyle, T.J.B., and E.K. Morgenstern. 1986. Estimates of outcrossing rates in six populations of black spruce in central New Brunswick. *Silvae Genet.* 35: 102-106.

Boyle, T.J.B., and E.K. Morgenstern. 1987. Some aspects of the population structure of black spruce in central New Brunswick. *Silvae Genet.* 36: 53-60.

Boyle, T.J.B., J.J. Balatinecz and P.M. McCaw. 1988. Genetic control of some wood properties in black spruce. Pp 198 *in* E.K. Morgensternand T.J.B. Boyle (eds.), Proc. 21st Can. Tree Impr. Assoc., Truro, N.S., Part 2, 213 pp.

Boyle, T.J.B., C. Liengsiri and C. Piewluang. 1990. Genetic structure of black spruce on two contrasting sites. *Heredity* 65: 393-399.

Braekke, F.H. 1990. Performance of *Picea mariana* in Norway. Pp 97-106 *in* B.D.Titus, M.B. Lavigne, P.F. Newton and W.J. Meades (eds.), The silvics and ecology of boreal spruces. 1989 IUFRO Working Party S1.05-12 Symp. Proc. Newfoundland, 12-17 Aug. 1989. For. Canada Inf. Rep. N-X-271.

Brockerhoff, E.G., and R.H. Ho. 1997. Effects of gibberellin $A_{4/7}$ stem injection on seed cone production in mature black spruce. *Can. J. For. Res.* 27: 1325-1328.

Brown, G. 1979. A black spruce gene pool for the northwestern region. Pp 202-207 *In* J. Scarratt, (coord.), Tree improvement symposium. Great Lakes For. Res. Centre COJFRC Symposium Proc. O-P-7, 236 pp.

Campbell, C.S., W.A. Wright, M. Cox, T.F. Vining, C.S. Major and M.P. Arsenault. 2005. Nuclear ribosomal DNA internal transcribed spacer 1 (ITS1) in *Picea* (Pinaceae): sequence divergence and structure. *Molec. Phylogenetics and Evol.* 35: 165-185.

Canadian Council of Forest Ministers. 2002. National Forestry Database Program. Online at: http://nfdp.ccfm.org/.

Carleton, T.J. 1982. The pattern of invasion and establishment of *Picea mariana* (Mill.) B.S.P. into the subcanopy layers of *Pinus banksiana* Lamb. dominated stands. *Can. J. For. Res.* 12: 973-984.

Caron, G-E. 1994. Seasonal variation in pollen catch in a black spruce orchard. *Grana* 33: 313-320.

Caron, G.E., and G.R. Powell. 1989. Patterns of seed-cone and pollen-cone production in young *Picea mariana* trees. *Can. J. For. Res.* 19: 359-364.

Caron, G.E., and G.R. Powell. 1992. Patterns of cone distribution in crowns of young *Picea mariana.* I. Effect of tree age on seed cones. *Can. J. For. Res.* 22: 46-55.

Carter, K.K., D.H. DeHayes, M.E. Demeritt Jr., R.T. Eckert , P.W. Garrett, H.D. Gerhold, J.E. Kuser and K.C. Steiner. 1988. Tree Improvement in the Northeast: interim summary and recommendations for selected species. Univ. Maine Agric. Exper. Stat. Tech. Bull. 131, 50 pp.

Chai, T.S., and H.L. Hansen. 1952. Characteristics of black spruce seed from cones of different ages. Univ. Minn. Sch. For., Minn. For. Notes 2, 2 pp.

Chang, J., and J.W. Hanover. 1991. Geographic variation in the monoterpene composition of black spruce. *Can. J. For. Res.* 21: 1796-1800.

Cheliak, W.M., and D.L. Rogers. 1990. Integrating biotechnology into tree improvement programs. *Can. J. For. Res.* 20: 452-463.

Cheliak, W.M., and K. Klimaszewska. 1991. Genetic variation in somatic embryogenic response in open-pollinated families of black spruce. *Theor. Appl. Genet.* 82: 185-190.

Cherry, M.L., and D.G. Joyce. 1998. Implementation of a second generation breeding program for black spruce in Ontario. Abstract 269 *in* Frontiers of Forest Biology, Proc. joint meeting of the N.A. For. Biol. Workshop / Western For. Genetics Assoc., Victoria, B.C.

Cogbill, C.V. 1985. Dynamics of the boreal forest of the Laurentian Highlands. *Can. J. For. Res.* 15: 252-261.

Colombo, S.J., M.L. Colclough, V.R. Timmer and E. Blumwald. 1992. Clonal variation in heat tolerance and heat shock protein expression in black spruce. *Silvae Genet.* 41: 234-239.

Conkle, M.T. 1992. Genetic diversity - seeing the forest through the trees. *New For.* 6: 5-22.

Côté, M., J. Ferron and R. Gagnon. 2003. Impact of seed and seedling predation by small rodents on early regeneration establishment of black spruce. *Can. J. For. Res.* 33: 2362-2371.

Côté, M., J. Ferron and R. Gagnon. 2005. Invertebrate predation of postdispersal seeds and juvenile seedlings of black spruce (*Picea mariana*) in the boreal forest of eastern Canada. *Can. J. For. Res.* 35: 674-681.

Critchfield, W.B. 1984. Impact of the Pleistocene on the genetic structure of North American conifers. Pp 70-118 in R.M. Lanner (ed.), Symposium on historical and genetic components of geographic variation patterns. Proc., 8th N.A. For. Biol. Workshop, Utah, 196 pp.

Dallimore, W., and A.B. Jackson. 1948. A handbook of Coniferae including Ginkgoaceae. Edward Arnold and Co., London, Great Britain, 682 pp.

den Ouden, P., and B.K. Boom. 1982. Manual of cultivated conifers hardy in the cold- and warm-temperate zone, 3rd ed. Martinus Nijhoff, the Hague, 520 pp.

Desponts, M., and J-P. Simon. 1987. Genetic structure and variability in populations of black spruce (*Picea mariana* [Mill.] B.S.P.) in the subarctic region of northern Québec. *Can. J. For. Res.* 17: 1006-1012.

Desponts, M., and S. Payette. 1993. The Holocene dynamics of jack pine at its northern range limit in Québec. *J. Ecol.* 81: 719-727.

deWitt, J. 1990a. Cultural advances towards a superior clonal plug at the Northern Clonal Forestry Centre. P. 18-20 *in* D.L. Rogers (ed.), Proceedings, annual meeting of the black spruce clonal forestry program. OMNR Northern For. Devel. Group, 59 pp.

deWitt, J. 1990b. Tour of Northern Clonal Forestry Centre. Pp. 18-20 *in* D.L. Rogers (ed.), Proceedings, annual meeting of the black spruce clonal forestry program. OMNR Northern For. Devel. Group, 59 pp.

Di-Giovanni, F., P.G. Kevan and J. Arnold. 1996. Lower planetary boundary layer profiles of atmospheric conifer pollen above a seed orchard in northern Ontario, Canada. *For. Ecol. Manag.* 83: 87-97.

Duchaufour, P., and L.Z. Rousseau. 1959. Poisoning of seedlings of conifers by the manganese in forest humus. *Rev. For. Franç.* 11: 835-847.

Dugle, J.R., and N. Bols. 1971. Variation in *Picea glauca* and *P. mariana* in Manitoba and adjacent areas. Atomic Energy of Canada Ltd., Whiteshell Nuclear Res. Establ., Pinawa, Man. Publ. AECL-3681, 63 pp.

Eckert, R.T. 1989. Genetic variation in red spruce and its relation to forest decline in the northeastern United States. Pp. 319-324 *in* J.B. Bucherand I. Bucher-Wallin (eds.), Proc. 14th Int'l. Meeting for Specialists in Air Pollution Effects on Forest Ecosystems, IUFRO P2.05, Interlaken, Switzerland, Oct. 2-8, 1988.

Elliot, K.R. 1960. A history of recent infestations of the spruce budworm in north-western Ontario, and an estimate of resultant timber losses. *For. Chron.* 36: 61-82.

Ellstrand, N.C. 1992. Gene flow among seed plant populations. *New For.* 6: 241-256.

Eriksson, G., G. Namkoong and J.H. Roberds. 1993. Dynamic gene conservation for uncertain futures. *For. Ecol. Manag.* 62: 15-37.

Farrar, J.L. 1995. Trees in Canada. Fitzhenry & Whiteside Ltd. / Natural Resources Canada, Canadian Forest Service, Markham / Ottawa, Ont., 502 pp.

Florin, R. 1963. The distribution of conifer and taxad genera in time and space. *Acta Horti Bergiani* 20: 121-312.

Foster, D.R. 1985. Vegetation development following fire in *Picea mariana* (black spruce)-*Pleurozium* forests of south-eastern Labrador, Canada. *J. Ecol.* 73: 517-534.

Fowells, H.A. (compiler). 1965. Silvics of forest trees of the United States. USDA For. Serv. Agric. Handbook No. 271.

Fowler, D.P. 1980. Hybridization of black spruce and Serbian spruce. Canadian Forest Service Marit. For. Res. Centre Info Rep. M-X-112, 30 pp.

Fowler, D.P. 1983. The hybrid black x Sitka spruce, implications to phylogeny of the genus *Picea*. *Can. J. For. Res.* 13: 108-115.

Fowler, D.P. 1986. Strategies for the genetic improvement of important tree species in the Maritimes. Can. For. Serv. Marit. Info Rep. M-X-156, 30 pp.

Fowler, D.P., and R.E. Mullin. 1977. Upland-lowland ecotypes not well developed in black spruce in northern Ontario. *Can. J. For. Res.* 7: 35-40.

Fowler, D.P., and Y.S. Park. 1982. Range-wide black spruce provenance trials in the Maritimes. CFS Marit. For. Res. Centre Info Rep. M-X-137, 25 pp.

Fowler, D.P., H.G. MacGillivray, S.A. Manley and J.M. Bonga. 1971. Tree breeding at the Maritimes Forest Research Centre, 1970. Pp. 23-29 *in* Proc. 13th Meeting of Comm. on Forest Tree Breeding in Canada, Prince George, B.C., Part 1, 142 pp.

Fowler, D.P., H.G. MacGillivray, S.A. Manley and J.M. Bonga. 1973. Tree breeding at the Maritimes Forest Research Centre, 1971 and 1972. Pp. 21-28 *in* Proc. 14th Can. Tree Impr. Assoc., Fredericton, N.B., Part 1, 200 pp.

Fowler, D.P., J.M. Bonga, J.F. Coles, Y.S. Park and R.F. Smith. 1979. Tree breeding at the Maritimes Forest Research Centre, 1977 and 1978. Pp. 81-87 *in* Proc. 17th Can. Tree Impr. Assoc., Gander, Newfoundland, Part 1, 256 pp.

Fraser, J.W. 1976. Viability of black spruce seed in or on a boreal forest seedbed. *For. Chron.* 52: 229-231.

Gajewski, K., S. Payette and J.C. Ritchie. 1993. Holocene vegetation history at the boreal forest - shrub-tundra transition in north-western Québec. *J. Ecol.* 81: 433-443.

Gamache, I., J.P. Jaramillo-Correa, S. Payette, and J. Bousquet. 2003. Diverging patterns of mitochondrial and nuclear DNA diversity in subarctic black spruce: imprint of a founder effect associated with postglacial colonization. *Molec. Ecol.* 12: 891-901.

Germano, J., and A.S. Klein. 1999. Species-specific nuclear and chloroplast single nucleotide polymorphisms to distinguish *Picea glauca*, *P. mariana* and *P. rubens*. *Theor. Appl. Genet.* 99:37-49.

Goff, H.L., and L. Sirois. 2004. Black spruce and jack pine dynamics simulated under varying fire cycles in the northern boreal forest of Quebec, Canada. *Can. J. For. Res.* 34: 2399-2409.

Gordon, A.G. 1975. Genetics and racial variation in *Picea*. Pp 77-79 *in* Proc. 15th Can. Tree Impr. Assoc., Chalk River, Ont., Part 1, 204 pp.

Gordon, A.G. 1976. The taxonomy and genetics of *Picea rubens* and its relationship to *Picea mariana*. *Can. J. Bot.* 54: 781-813.

Gordon, A.G. 1979. Spruce genetics, Sault Ste. Marie in 1977 and 1978. Pp 117-121 *in* Proc. 17th Can. Tree Impr. Assoc., Gander, Newfoundland, Part 1, 256 pp.

Gordon, A.G. 1981. Genetics and genecology of spruce, Sault Ste. Marie, Ont. 1979 and 1980. Pp 112-115 *in* Proc. 18th Can. Tree Impr. Assoc., Duncan, British Columbia, Part 1, 231 pp.

Gordon, A.G. 1985. Breeding, genetics and genecological studies in spruce for tree improvement in 1983 and 1984, Sault Ste. Marie, Ontario Pp 112-116 *in* Proc. 20th Can. Tree Impr. Assoc., Québec City, Part 1, 269 pp.

Govindaraju, D.R. 1988. Relationship between dispersal ability and levels of gene flow in plants. *Oikos* 52: 31-35.

Greene, D.F., J. Noël, Y. Bergeron, M. Rousseau and S. Gauthier. 2004. Recruitment of *Picea mariana*, *Pinus banksiana*, and *Populus tremuloides* across a burn severity gradient following wildfire in the southern boreal forest of Quebec. *Can. J. For. Res.* 34: 1845-1857.

Greenwood, M.S., G.W. Adams and M. Gillespie. 1988. Shortening the breeding cycle of some northeastern conifers. Pp 43-52 *in* E.K. Morgenstern and T.J.B.Boyle (eds.), Proc. 21st Can. Tree Impr. Assoc., Truro, N.S., Part 2, 213 pp.

Groot, A., and B.J. Horton. 1994. Age and size structure of natural and second-growth peatland *Picea mariana* stands. *Can. J. For. Res.* 24: 225-233.

Haavisto, V.F. 1975. Peatland black spruce seed production and dispersal in northeastern Ontario. Pp 250-264 *in* Black Spruce Symposium. Canadian Forest Service, Great Lakes For. Res. Centre, Sympos. Proc. O-P-4.

Haavisto, V.F. 1978. Lowland black spruce seedfall: viable seedfall peaks in mid-April. *For. Chron.* 54:213-215.

Hakman, I., and L.C. Fowke. 1987. Somatic embryogenesis in *Picea glauca* (white spruce) and *Picea mariana* (black spruce). *Can. J. Bot.* 65: 656-659.

Hall, J.P. 1986. Regional provenance trials of black spruce in Newfoundland: fifteen years from seed. Newfoundland Forest Centre Info Rep. N-X-255, 22 pp.

Hamel, B., N. Bélanger and D. Paré. 2004. Productivity of black spruce and Jack pine stands in Quebec as related to climate, site biological features and soil properties. *For. Eco. Man.* 191: 239-251.

Harper, K.A., Y. Bergeron, S. Gauthier and P. Drapeau. 2002. Post-fire development of canopy structure and composition in black spruce forests of Abitibi, Québec: A landscape scale study. *Silva Fenn.* 36(1): 249-263.

Harper, K., C. Boudreault, L. DeGrandpré, P. Drapeau, S. Gauthier and Y. Bergeron. 2003. Structure, composition, and diversity of old-growth black spruce boreal forest of the Clay Belt region in Quebec and Ontario. *Environ. Rev.* 11: S79-S98.

Hatcher, R.J. 1963. A study of black spruce forest in northern Québec. Dept. of For. Can., For. Res. Br., Pub. No. 1018, 37 pp.

Hawley, G.J., and D.H. DeHayes. 1994. Genetic diversity and population structure of red spruce (*Picea rubens*). *Can. J. Bot.* 72: 1778-1786.

Hawley, G.J., D.H. DeHayes and G.J. Badger. 2000. Red and black spruce introgression in montane ecosystems in New England and New York. *J. Sustain. For.* 10: 327-333.

Heinselman, M.L. 1957. Silvical characteristics of black spruce. USDA For. Serv. Lake States For. Exp. Stat. Paper 45, 30 pp.

Hillier, H.G. 1981. Hilliers manual of trees and shrubs, 5th ed. David and Charles Publ. Ltd., 575 pp.

Hills, L.V., and R.T. Ogilvie. 1970. *Picea banksii* n. sp. Beaufort Formation (Tertiary), Northwestern Banks Island, Arctic Canada. *Can. J. Bot.* 48: 457-464.

Ho, R.H. 1991. A guide to pollen- and seed-cone morphology of black spruce, jack pine and eastern white pine for controlled pollination. Min. Nat. Res. Ont. For. Res. Instit. For. Res. Rep. No. 125, 31 pp.

Hodgetts, R.B., M.A. Aleksiuk, A. Brown, C. Clarke, E. MacDonald, S. Nadeem and D. Khasa. 2001. Development of microsatellite markers for white spruce (*Picea glauca*) and related species. *Theor. Appl. Genet.* 102: 1252-1258.

Hopkin, A., T. Dumond and R.J. Sajan. 1994a. Incidence of pests in black spruce plantations in Ontario. Natural Resources Canada, Canadian Forest Service, Sault Ste. Marie, Ontario. Frontline Tech. Note 79, 4 pp.

Hopkin, A., R. Sajan and T. Dumond. 1994b. Incidence of pests in black spruce seed orchards in Ontario. Natural Resources Canada, Canadian Forest Service, Sault Ste. Marie, Ontario. Frontline Tech. Note 80, 4 pp.

Horton, K.W., and J.C. Lees. 1961. Black spruce in the foothills of Alberta. Can. Dept. For. Res. Br. Tech. Note No. 110, 54 pp.

Howard, F.W. 1962. Establishment report: black spruce seed trapping experiment, south-west Gander Watershed, Newfoundland. Can. Dept. For., For. Res. Br. Mimeo, Newfoundland. 62-5.

Isabel, N., J. Beaulieuand and J. Bousquet. 1995. Complete congruence between gene diversity estimates derived from genotypic data at enzyme and random amplified polymorphic DNA loci in black spruce. *Proc. Natl. Acad. Sci. U.S.Am.* 92: 6369-6373.

Ives, W.G.H., and H.R. Wong. 1988. Tree and shrub insects of the prairie provinces. For. Can., Northern Reg., Edmonton, AB. Info. Rep. NOR-X-292, 327 pp.

Jablanczy, A. 1964. Identification of black, red and white spruce seedlings. Dept. For. Publ. No. 1039, 11 pp.

Jacobson, G.L. Jr., T. Webb III and E.C. Grimm. 1987. Patterns and rates of vegetation change during the deglaciation of eastern North America. Pp. 277-288 *in* W.F. Ruddiman and J.E. Wright Jr. (eds.), North America and Adjacent Oceans During the Last Deglaciation. Geogr. Soc. Amer., Boulder, Co., The Geology of North America K-3, 501 pp.

Jaramillo-Correa, J.P., and J. Bousquet. 2003. New evidence from mitochondrial DNA of a progenitor-derivative species relationship between black spruce and red spruce (Pinaceae). *Am. J. Bot.* 90(12): 1801-1806.

Jaramillo-Correa, J.P., J. Beaulieu and J. Bousquet. 2004. Variation in mitochondrial DNA reveals multiple distant glacial refugia in black spruce (*Picea mariana*), a transcontinental North American conifer. *Molec. Ecol.* 13: 2735-2747.

Johnsen, K.H., and J.E. Major. 1998. Black spruce family growth performance under ambient and elevated atmospheric CO_2. *New For.* 15: 271-281.

Johnsen, K.H., J.R. Seilerand and J.E. Major. 1996. Growth, shoot phenology, and physiology of diverse seed sources of black spruce: II. 23-year-old trees. *Tree Physiol.* 16: 375-380.

Johnsen, K.H., J.E. Major, J. Loo and D. McPhee. 1998. Negative heterosis not apparent in 22-year-old hybrids of *Picea mariana* and *Picea rubens. Can. J. Bot.* 76: 434-439.

Johnsen, K.H., L.B. Flanagan, D.A. Huber and J. Major. 1999. Genetic variation in growth, carbon isotope discrimination, and foliar N concentration in *Picea* mariana: analyses from a half-diallel mating design using field-grown trees. *Can. J. For. Res.* 29: 1727-1735.

Johnsen, K., J.E. Major and C.A. Maier. 2003. Selfing results in inbreeding depression of growth but not of gas exchange of surviving adult black spruce trees. *Tree Physiol.* 23: 1005-1008.

Johnson, H. J. 1956. Some aspects of black spruce reproduction in the central boreal forest region of Manitoba. Dept. of Northern Affairs and Natural Resources, For. Br., For. Res. Div., Project Manuscr. 175, 15 pp.

Johnston, W.F. 1977. Manager's handbook for black spruce in north central states. USDA For. Serv. North Central For. Exper. Stat. Gen. Tech. Rep. NC-34, 18 pp.

Johnston, W.F., and T.M. Smith. 1983. Black spruce. Pp 96-98 *in* R.M. Burns(ed.), Silvicultural systems for major forest types of the United States. USDA For. Serv. Agric. Handbook no. 445, 193 pp.

Johnstone, J.F., F.S. Chapin III, J. Foote, S. Kemmett, K. Price and L. Viereck. 2004. Decadal observations of tree regeneration following fire in boreal forests. *Can. J. For. Res.* 34: 267-273.

Khalil, M.A.K. 1975. Genetic variation in black spruce (*Picea mariana* [Mill.] B.S.P.) in Newfoundland. *Silvae Genet.* 24: 88-96.

Khalil, M.A.K. 1984a. All-range black spruce provenance study in Newfoundland: performance and genotypic stability of provenances. *Silvae Genet.* 33: 63-71.

Khalil, M.A.K. 1984b. Genetics of cone morphology of black spruce (*Picea mariana* [Mill.] B.S.P.) in Newfoundland, Canada. *Silvae Genet.* 33: 101-109.

Khalil, M.A.K. 1985. Genetics of wood characters of black spruce (*Picea mariana* [Mill.] B.S.P.) in Newfoundland, Canada. *Silvae Genet.* 34: 221-230.

Khalil, M.A.K. 1986. Variation and genotypic stability of *Picea mariana* [Mill.] B.S.P. in Newfoundland, Canada. *Silvae Genet.* 35: 49-57.

Khalil, M.A.K., and A.W. Douglas. 1979. Correlation of height growth in black spruce with site factors of the provenances. Silvae Genet. 28: 122-124.

Kleinschmidt, J. 1992. Use of spruce cuttings in plantations. Pp 1-10 *in* D.A. Rook (ed.), Super Sitka for the 90s. For. Comm. Bull. 103, 75 pp.

Klimaszewska, K., C. Ward and W.M. Cheliak. 1992. Cryopreservation and plant regeneration from embryonic cultures of larch (*Larix* x *eurolepis*) and black spruce (*Picea mariana*). *J. Exp. Bot.* 43: 73-79.

Klimaszewska, K., D. Lachance, M. Bernier-Cardou and R.G. Rutledge. 2003. Transgene integration patterns and expression levels in transgenic tissue lines of *Picea mariana*, *P. glauca* and *P. abies*. *Plant Cell Rep.* 21: 1080-1087.

Knowles, P. 1985. Comparison of isozyme variation among natural stands and plantations: jack pine and black spruce. *Can. J. For. Res.* 15: 902-908.

Knowles, P. 1991. Spatial genetic structure within two natural stands of black spruce (*Picea mariana* [Mill.] B.S.P.). *Silvae Genet.* 40: 13-19.

Koubaa A., S.Y. Zhang, N. Isabel, J. Beaulieu and J. Bousquet. 2000. Phenotypic correlations between juvenile-mature wood density and growth in black spruce. *Wood and Fiber Science.* 32: 61-71.

Krause, C., and H. Morin. 1999. Tree-ring patterns in stems and root systems of black spruce (*Picea mariana*) caused by spruce budworm. *Can. J. For. Res.* 29: 1583-1591.

Landhäusser, S.M., and R.W. Wein. 1993. Postfire vegetation recovery and tree establishment at the Arctic treeline: climate-change-vegetation-response hypotheses. *J. Ecol.* 81: 665-672.

Larocque, I., Y. Bergerson, I.D. Campbell and R.H.W. Bradshaw. 2000. Vegetation changes through time on islands of Lake Duparquet, Abitibi, Canada. *Can. J. For. Res.* 30: 179-190.

Lavoie, C., and S. Payette. 1992. Black spruce growth forms as a record of a changing winter environment at treeline, Québec, Canada. *Arct. Alp. Res.* 24: 40-49.

Lavoie, L., and L. Sirois. 1998. Vegetation changes caused by recent fires in the northern boreal forest of eastern Canada. *J. Veg. Sci.* 9: 483-492.

Larsen, J.A. 1965. The vegetation of Ennadai Lake Area, N.W.T.: studies in arctic and subarctic bioclimatology. *Ecol. Monogr.* 35: 37-59.

LeBarron, R.K. 1944. Influences of controllable environmental conditions on regeneration of jack pine and black spruce. *J. Agric. Res.* 68: 97-119.

LeBarron, R.K. 1948. Silvicultural management of black spruce in Minnesota. USDA Wash. D.C. Circular No. 791, 60 pp.

Legere, A. and S. Payette. 1981. Ecology of a black spruce (*Picea mariana*) clonal population in the hemiarctic zone, northern Quebec: Population dynamics and spatial development. *Arct. Alp. Res.* 13: 261-276.

Li, H.L. 1953. Present distribution and habitats of the conifers and taxads. *Evolution* 7: 245-261.

Lieffers, V.J. 1986. Stand structure, variability in growth and intraspecific competition in a peatland stand of black spruce *Picea mariana*. *Holarct. Ecol.* 9: 58-64.

Little, E.L. Jr. 1971. Atlas of United States trees, volume 1, conifers and important hardwoods. USDA Misc. Publ. v. 1146, 9 pp + 200 maps.

Little, E.L., and S.S. Pauley. 1958. A natural hybrid between black and white spruce in Minnesota. *Am. Midl. Nat.* 60: 202-211.

Livingston, W.H. 1990. *Armillaria ostoyae* in young spruce plantations. *Can. J. For. Res.* 20: 1773-1778.

Lulsdorf, M.M., T.E. Tautorus, S.I. Kikcio, T.D. Bethune and D.I. Dunstan. 1993. Germination of encapsulated embryos of interior spruce (*Picea glauca engelmannii* complex) and black spruce (*Picea mariana* Mill.). *Plant Cell Rep.* 12: 385-389.

Lussier, J.-M., H. Morin and R. Gagnon. 1992. Comparaison de la croissance de marcottes d'épinette noire (*Picea mariana*) adultes après coupe à celle d'individus issus de graines après feu. *Can. J. For. Res.* 22: 1524-1535.

MacAloney, H.J. 1936. The European spruce sawfly in the United States. *J. For.* 34: 125-129.

Major, J.E., A. Mosseler, D.C. Barsi, M. Campbell and O.P. Rajora. 2003. Morphometric, allometric, and developmentally adaptive traits in red spruce and black spruce. II. Seedling and mature tree assessment of controlled intra- and inter-specific hybrids. *Can. J. For. Res.* 33: 897-909.

Major, J.E., A. Mosseler, K.H. Johnsen, O.P. Rajora, D.C. Barsi, K-H. Kim, J-M. Park and M. Campbell. 2005. Reproductive barriers and hybridity in two spruces, *Picea rubens* and *Picea mariana*, sympatric in eastern North America. *Can. J. Bot.* 83: 163-175.

Maliondo, S.M., and H.H. Krause. 1985. Genotype and soil fertility interaction in the growth of black spruce progeny from a central New Brunswick population. *Can. J. For. Res.* 15: 410-416.

Manley, S.A.M. 1971. Identification of red, black, and hybrid spruces. Canadian Forest Service Publ. No. 1301, 14 pp.

Manley, S.A.M. 1972. The occurrence of hybrid swarms of red and black spruces in central New Brunswick. *Can. J. For. Res.* 2: 381-391.

Manley, S.A.M., and D.P. Fowler. 1969. Spruce budworm defoliation in relation to introgression in red and black spruce. *For. Sci.* 15: 365-366.

Manley, S.A.M., and F.T. Ledig. 1979. Photosynthesis in black and red spruce and their hybrid derivatives: ecological isolation and hybrid adaptive inferiority. *Can. J. Bot.* 57: 305-314.

Marles, R.J., C. Clavelle, L. Monteleone, N. Tays and D. Burns. 2000. Aboriginal plant use in Canada's northwest boreal forest. Natural Resources Canada, Canadian Forest Service / UBC Press, Vancouver, 368 pp.

MacGillivray, H.G. 1955. Germination of spruce and fir seed following different stratification periods. *For. Chron.* 31: 365.

Martineau, R. 1984. Insects Harmful to Forest Trees. Multiscience Publications Limited and the Canadian Forest Service. Min. Supply and Services Canada. Ottawa, Ontario. 261 p.

Mercier, S., and P. Périnet. 1998. The second generation seed orchard research project at the Direction de la recherche forestière in Québec. *For. Chron.* 74: 181-184.

Merrill, R.E., C.A. Mohn and W. Cromwell. 1984. Survival and height growth of a rangewide black spruce test on two sites in Minnesota. Proc. 3rd North Central Tree Improvement Assoc. Meeting, Wooster, Ohio, 1983, Dept. Forestry, Univ. Wisc., pp. 34-43.

Mikkola, L. 1969. Observations on interspecific sterility in *Picea*. *Ann. Bot. Fenn.* 6: 285-339.

Mitchell, R.G., N.E. Johnson and K.H. Wright. 1974. Susceptibility of 10 spruce species and hybrids to the white pine weevil (=Sitka spruce weevil) in the Pacific Northwest. USDA For. Serv. Pacific Northwest For. Range Exp. Stat. Res. Note PNW-225, 8 pp.

Mitchell, R.G., K.H.Wright and N.E. Johnson. 1990. Damage by the Sitka spruce weevil (*Pissodes strobi*) and growth patterns for 10 spruce species and hybrids over 26 years in the Pacific Northwest. USDA For. Serv. Dept. Agric. Pacific Northwest Res. Stat. Res. Pap. PNW-RP-434, 12 pp.

Morgenstern, E.K. 1962. Note on chromosome morphology in *Picea rubens* Sarg. and *Picea mariana* (Mill.) B.S.P. *Silvae Genet.* 11: 163-164.

Morgenstern, E.K. 1969a. Genetic variation in seedlings of *Picea mariana* (Mill.) B.S.P. I. Correlation with ecological factors. *Silvae Genet.* 18: 151-161.

Morgenstern, E.K. 1969b. Genetic variation in seedlings of *Picea mariana* (Mill.) B.S.P. II. Variation patterns. *Silvae Genet.* 18: 161-167.

Morgenstern, E.K. 1972. Preliminary estimates of inbreeding in natural populations of black spruce, *Picea mariana*. *Can. J. Genet. Cytol.* 14: 443-446.

Morgenstern, E.K. 1973. Genetic research with black and red spruce at Petawawa Forest Experiment Station. Canadian Forest Service Petawawa For. Exper. Stat. Info Rep. PS-X-45, 35 pp.

Morgenstern, E.K. 1974a. A diallel cross in black spruce, *Picea mariana* (Mill.) B.S.P. *Silvae Genet.* 23 : 67-70.

Morgenstern, E.K. 1974b. Early results of provenance hybridization in black spruce. Pp 104 *in* Proc. 21st Northeastern forest tree improvement conference, Fredericton, New Brunswick, 1973.

Morgenstern, E.K. 1975a. Early results of provenance hybridization in black spruce. Pp 27-37 *in* D.P. Fowler and C.W. Yeatman(eds.), Proceedings of the fourteenth meeting of the Canadian Tree Improvement Assoc., Fredericton, New Brunswick, 1973, 142 pp.

Morgenstern, E.K. 1975b. Effects of seed origin and their utilization through silviculture and tree breeding. Pp 107-120 *in* Anon. Black spruce symposium. Canadian Forest Service Great Lakes For. Res. Centre Symposium Proceedings O-P-4, 289 pp.

Morgenstern, E.K. 1996. Geographic variation in trees: genetic basis and application of knowledge in silviculture. UBC Press, Vancouver, 209 pp.

Morgenstern, E.K., and J.L. Farrar. 1964. Introgressive hybridization in red spruce and black spruce. Univ. Toronto Faculty of Forestry Tech. Rep. No. 4, 46 pp.

Morgenstern, E.K., and D.P. Fowler. 1969. Genetics and breeding of black spruce and red spruce. *For. Chron.* 45: 408-412.

Morgenstern, E.K., and T.J. Mullin. 1990. Growth and survival of black spruce in the range-wide provenance study. *Can. J. For. Res.* 20: 130-143.

Morgenstern, E.K., and Y.S. Park. 1991. Breeding of *Picea mariana* (Mill.) B.S.P.: seed orchard and clonal approaches. *Silva Fenn.* 25: 280-285.

Morin, H., and R. Gagnon. 1992. Comparative growth and yield of layer- and seed-origin black spruce (*Picea mariana*) in Québec. *Can. J. For. Res.* 22: 465-473.

Mullin, T.J. 1985. Genotype-nitrogen interactions in full-sib seedlings of black spruce. *Can. J. For. Res.* 15: 1031-1038.

Mullin, T.J., and Y.S. Park. 1994. Genetic parameters and age-age correlations in a clonally replicated test of black spruce after 10 years. *Can. J. For. Res.* 24: 2330-2341.

Mullin, T.J., G.W. Adams, J.D. Simpson, K.J. Tosh and M.S. Greenwood. 1995. Genetic parameters and correlations in tests of open-pollinated black spruce families in field and retrospective nursery test environments. *Can. J. For. Res.* 25: 270-285.

Myren, D., and A. Cameron. 1995. Diseases of black spruce in Ontario. Natural Resources Canada, Canadian Forest Service, Sault Ste. Marie, ON. Frontline Tech. Note 78, 4 pp.

Nealis, V.G., and J. Régnière. 2004. Insect-host relationships influencing disturbance by the spruce budworm in a boreal mixedwood forest. *Can. J. For. Res.* 34: 1870-1882.

Nelson, C.D., and C.A. Mohn. 1991. Genetic variance of early height growth and expected gains from selection in a Minnesota population of black spruce. *Can. J. For. Res.* 21: 11-19.

Nienstaedt, H. 1984. Breeding and juvenile selection in a range-wide black spruce provenance test. *Can. J. For. Res.* 14: 933-939.

Nienstaedt, H., and A. Teich. 1971. Genetics of white spruce. USDA For. Serv. Res. Pap. WO-15, 24 pp.

Nkongolo, K.K., and K. Klimaszewska. 1993. Karyotype analysis and optimization of mitotic index in *Picea mariana* (black spruce) preparations from seedling root tips and embryonic cultures. *Heredity* 73:11-17.

Nkongolo, K.K., L. Deverno and P. Michael. 2003. Genetic validation and characterization of RAPD markers differentiating black and red spruces: molecular certification of spruce trees and hybrids. *Plant Syst. Evol.* 236: 151-163.

Nkongolo, K.K., P. Michael and T. Demers. 2005. Application of ISSR, RAPD, and cytological markers to the certification of *Picea mariana, P. glauca,* and *P. engelmannii* trees, and their putative hybrids. *Genome* 48: 302-311.

O'Connell, K.E.B., S.T. Gower and J.M. Norman. 2003. Net ecosystem production of two contrasting boreal black spruce forest communities. *Ecosystems* 6: 248-260.

Ogilvie, R.T. 1972. Speciation in the North American spruces and its relation to white spruce. Pp 1-7 *in* R.G. McMinn(ed.), White spruce: the ecology of a northern resource. Canadian Forest Service Northern For. Res. Centre Info Rep. NOR-X-40, 44 pp.

O'Reilly, C., and W.H. Parker. 1982. Vegetative phenology in a clonal seed orchard of *Picea glauca* and *Picea mariana* in northwestern Ontario. *Can. J. For. Res.* 12: 408-413.

O'Reilly, C., W.H. Parker and J.E. Barker. 1982. Effect of pollination period and strobili number on random mating in a clonal seed orchard of *Picea mariana*. *Silvae Genet.* 31: 90-94.

O'Reilly, G.J., W.H. Parker and W.M. Cheliak. 1985. Isozyme differentiation of upland and lowland *Picea mariana* stands in northern Ontario. *Silvae Genet.* 34: 214-221.

Osawa, A. 1989. Causality in mortality patterns of spruce trees during a spruce budworm outbreak. *Can. J. For. Res.* 19: 632-638.

Owens, J.N., and M.D. Blake. 1985. Forest tree seed production. Canadian Forest Service Petawawa National Forest Institute Info Rep. PI-X-53, 161 pp.

Paquin, R., and R. Doucet. 1992. Croissance en hauteur à long terme de la régénération préétablie dans des pessières noires boréales régénerées par marcottage, au Québec. *Can. J. For. Res.* 22: 613-621.

Paquin, R., H.A. Margolis, R. Doucet and M.R. Coyea. 1999. Comparison of growth and physiology of layers and naturally established seedlings of black spruce in a boreal cutover in Québec. *Can. J. For. Res.* 29: 1-8.

Parish, R., and S.M. Thomson. 1994. Tree book: learning to recognize trees of British Columbia. Canada-B.C. Partnership Agreement on Forest Resource Development: FRDA II, 183 pp.

Parisien, M., and L. Sirois 2003. Distribution and dynamics of tree species across a fire frequency gradient in the James Bay region of Quebec. *Can. J. For. Res.* 33: 243-256.

Park, Y.S., and D.P. Fowler. 1984. Inbreeding in black spruce (*Picea mariana* [Mill.] B.S.P.): self-fertility, genetic load, and performance. *Can. J. For. Res.* 14: 17-21.

Park, Y.S., and D.P. Fowler. 1988. Geographic variation of black spruce tested in the Maritimes. *Can. J. For. Res.* 18: 106-114.

Park, Y.S., J.D. Simpson, G.W. Adams, E.K. Morgenstern and T.J. Mullin. 1993. An updated breeding strategy for black spruce (*Picea mariana* [Mill.] B.S.P.) in New Brunswick. Pp 41-54 *in* Y.S. Parkand G.W. Adams (eds.), Breeding strategies of important tree species in Canada: workshop proceedings. Natural Resources Canada Info Rep. M-X-186E, 54 pp.

Park, Y.S., G.W. Adams and T.J. Mullin. 1998. Incorporation of new information and technology in breeding and deployment strategies for black spruce. Pp 3-23 *in* S. Puri (ed.), Tree Improvement Applied Research and Technology Transfer. Science Publ., Inc., Enfield, NH, 324 pp.

Parker, W.H. 1992. Focal point seed zones: site-specific seed zone delineation using geographic information systems. *Can. J. For. Res.* 22: 267-271.

Parker, W.H., and D.G McLachlan. 1978. Morphological variation in white and black spruce: investigation of natural hybridization between *Picea glauca* and *Picea mariana. Can. J. Bot.* 56: 2512-2520.

Payette, S. 1974. Classification écologique des formes de croissance de *Picea glauca* (Moench.) Voss et de *Picea mariana* (Mill.) BSP. en milieux subarctiques et subalpins. *Nat. can.* 101: 893-903.

Perron, M., and J. Bousquet. 1997. Natural hybridization between black spruce and red spruce. *Mol. Ecol.* 6: 725-734.

Perron, M., A.J. Gordon and J. Bousquet. 1995. Species-specific RAPD fingerprints for the closely related *Picea mariana* and *P. rubens*. Theor. *Appl. Genet.* 91: 142-149.

Perron, M., D.J. Perry, C. Andalo and J. Bousquet. 2000. Evidence from sequence-tagged-site markers of a recent progenitor-derivative species pair in conifers. *Proc. Natl. Acad. Sci. U.S.Am.* 97: 11331-11336.

Perry, D.J., and J. Bousquet. 1998. Sequence-tagged-site (STS) markers of arbitrary genes: development, characterization and analysis of linkage in black spruce. *Genetics* 149: 1089-1098.

Perry, D.J., and J. Bousquet. 2001. Genetic diversity and mating system of post-fire and post-harvest black spruce: an investigation using codominant sequence-tagged-site (STS) markers. *Can. J. For. Res.* 31: 32-40.

Pham, A.T., L. De Grandpré, S. Gauthier and Y. Bergeron. 2004. Gap dynamics and replacement patterns in gaps of the northeastern boreal forest of Quebec. *Can. J. For. Res.* 34: 353-364.

Pharis, R.P., F.C. Yeh and B.P. Dancik. 1991. Superior growth potential in trees: what is its basis, and can it be tested for at an early age? *Can. J. For. Res.* 21: 368-374.

Pitel, J.A., W.M. Cheliak and J. Barrett. 1987. Inheritance of allozymes in a black spruce diallel cross. *Silvae Genet.* 36: 149-153.

Place, I.C.M. 1955. The influence of seed-bed conditions on the regeneration of spruce and balsam fir. Can. Dept. Northern Affairs and Nat. Res. For. Br. Bull. 117, 87 pp.

Pollard, D.F.W., and K.T. Logan. 1974. The role of free growth in the differentiation of provenances of black spruce *Picea mariana* [Mill.] B.S.P. *Can. J. For. Res.* 4: 308-311.

Prager, E.M., D.P. Fowler and A.C. Wilson. 1976. Rates of evolution in conifers (*Pinaceae*). *Evolution* 30: 637-649.

Prévost, Y.H. 1990. Environmental architecture - preventing loss of seed production to insects in black and white spruce seed orchards. Pp. 100-117 *in* R.J. West (ed.) Proc. - Cone and Seed Pest Workshop, St. John's, Newfoundland 1989. For. Can. Info Rep. N-X-274, 128 pp.

Prévost, Y.H., J.E. Laing and V.F. Haavisto. 1988. Seasonal damage by insects and squirrels to female reproductive structures of black spruce, *Picea mariana* (Mill.) B.S.P. *Can. Entomol.* 120: 1113-1121.

Rajora, O.P. and S.A. Pluhar. 2004. Genetic diversity impacts of forest fires, forest harvesting, and alternative reforestation practices in black spruce (*Picea mariana*). *Theor. Appl. Genet.* 106: 1203-1212.

Raske, A.G., and W.J. Sutton. 1986. Decline and mortality of black spruce caused by spruce budworm defoliation and secondary organisms. Canadian Forest Service, Newfoundland Forestry Centre, Info. Rep. N X-236, 29 pp.

Rauter, R.M. 1971. Spruce program at the Southern Research Station, Maple, Ontario in 1970. Pp 81-84 *in* Proc. 13th Meeting of Comm. on Forest Tree Breeding in Canada, Prince George, B.C., Part 1, 142 pp.

Reeks, W.A., and G.W. Barter. 1951. Growth reduction and mortality of spruce caused by the European spruce sawfly, *Gilpinia hercyniae* (Htz.) (*Hymenoptera Diprionidae*). *For. Chron.* 27: 140-156.

Richardson, J. 1981. Black spruce research by the Canadian Forest Service in Newfoundland. Canadian Forest Service Info Rep. N-X-206.

Riemenschneider, D., and C.A. Mohn. 1975. Chromatographic analysis of an open-pollinated Rosendahl spruce progeny. *Can. J. For. Res.* 5: 414-418.

Robinson, F.C. 1974. A silvicultural guide to the black spruce Working Group *Picea mariana* (Mill.) BSP. Ontario Min. of Nat. Res., Div. of Forests, Forest Management Branch, 39 pp.

Roche, L. 1968. Introgressive hybridization in the spruce species of British Columbia. Pp. 249-270 *in* C.W. Yeatman (ed.), Proc. 11th Meeting of the Committee on Forest Tree Breeding in Canada, Part 2. Aug. 8-10, 1968, 290 pp.

Roche, L. 1969. A genecological study of the genus *Picea* in British Columbia. *New Phytol.* 68: 505-554.

Roche, L., and D.P. Fowler. 1975. Genetics of Sitka spruce. USDA For. Serv. Res. Pap. WO-26, 15 pp.

Roe, E.I. 1949. Sphagnum moss retards black spruce regeneration. USFS Lake States For. Exp. Stat. Tech. Note 321, 1 pp.

Rogers, D.L., and T.J.B. Boyle. 1991. Unequal paternal contributions in black spruce polycross seedlots. *Heredity* 67: 373-379.

Rudolf, P.O. 1965. Botanical and commercial range of black spruce in the Lake States. USDA For. Serv. Lake States For. Exper. Stat. Res. Note LS-74, 4 pp.

Safford, L.O. 1974. *Picea* A. Dietr. Spruce. Pp 587-597 *in* C.S. Schopmeyer (tech. coord.) Seeds of Woody Plants in the U.S. USDA For. Serv. Agric. Handbook No. 450, 883 pp.

Sargent, C.S. 1898. The silva of North America, Vol. 12. Houghton Mifflin, Boston. 144 pp.

Schooley, H.O. 1980. Damage to black spruce cone crops by the spruce budworm. Env. Can. For. Serv., Newf. For. Res. Cen., St. John's, Newf. Info. Rep. N-X-187.

Segaran, S. 1979. Comparative genotypic stability parameters in provenance selection as applied to twenty local provenances of black spruce (*Picea mariana* [Mill.] B.S.P.). Manit. Dept. Renew. Res. and Transport. Serv. Tree Impr. and For. Gen. Prog. MS Rep. 79-4, 66 pp.

Segaran, S., S. Monchak and C. North. 1978. Local provenance trials of black spruce (*Picea mariana* [Mill.] B.S.P.) in Manitoba. Man. Dept. Renew. Res. and Transport. Serv. Tree Impr. and For. Gen. Sect. Manuscr. Rep. 78-15, 34 pp.

Simpson, J.D. 1997. The New Brunswick tree improvement program. *J. For.* 95: 12-14.

Simpson, J.D., and G.R. Powell. 1981. Some factors influencing cone production on young black spruce in New Brunswick. *For. Chron.* 57: 267-269.

Simpson, J.D., and K. Tosh. 1997. The New Brunswick Tree Improvement Council is 20 years old. *For. Chron.* 73: 572-577.

Simpson, J.D., and B. Daigle. 1998. National Forest Genetic Resources Centre. Pp 47-48 *in* J.D. Simpson (ed.), Proc. 26th meeting of the Can. Tree Impr. Assoc., Ste.-Foy, Québec., Part 1, 159 pp.

Simpson, J.D., B.S.P. Wang and B.I. Daigle. 2004. Long-term seed storage of various Canadian hardwoods and conifers. *Seed Sci. and Technol.* 32: 561-572.

Sims, R., and S. Walsh. 1995. Black spruce advance growth: an important basis for stand renewal. Natural Resources Canada, Canadian Forest Service, Sault Ste. Marie, ON. Frontline Tech. Note 54, 4 pp.

Sirois, L. 1993. Impact of fire on *Picea mariana* and *Pinus banksiana* seedlings in subarctic lichen woodlands. *J. Veg. Sci.* 4: 795-802.

Sirois, L., and S. Payette. 1989. Postfire black spruce establishment in subarctic and boreal Quebec. *Can. J. For. Res.* 19: 1571-1580.

Skeates, D.A., and V.F. Haavisto. 1987. Black spruce cone and seed production: yields from Ontario collections. Ont. Min. Nat. Res. For. Res. Tree Impr. and For. Biomass Instit. Rep. No. 119, 16 pp.

Smith, D.E., and A.S. Klein. 1994. Phylogenetic inferences on the relationship of North American and European *Picea* species based on nuclear ribosomal 18S sequences and the internal transcribed spacer 1 region. *Mol. Phylogenet. Evol.* 3: 17-26.

Spies, C.J. III, and J.B. Dimond. 1985. Some populational relationships between spruce budworm, *Choristoneura fumiferana* (Lepidoptera Tortricidae) and spruce coneworm, *Dioryctria reniculelloides* (Lepidoptera: Pyralidae). *Can. Entomol.* 117: 193-202.

Sproule, A.T., and B.P. Dancik. 1996. The mating system of black spruce in north-central Alberta, Canada. *Silvae Genet.* 45: 159-164.

Stanek, W. 1961. Natural layering of black spruce in Northern Ontario. *For. Chron.* 37: 245-258.

Stanek, W. 1968. Development of black spruce layers in Northern Ontario. *For. Chron.* 44: 25-28.

Stanek, W. 1975. The role of layerings in black spruce forests on peatlands in the clay belt of northern Ontario. Pp 242-249 *in* Black Spruce Symposium. Canadian Forest Service, Dept. of the Envir. Sympos. Proc. O-P-4. 289 pp + appendix.

Stoehr, M.U., and R.E. Farmer Jr. 1986. Genetic and environmental variance in cone size, seed yield, and germination properties of black spruce clones. *Can. J. For. Res.* 16: 1149-1151.

St-Pierre, H., R. Gagnon and P. Bellefleur. 1992. Régénération après feu de l'épinette noire (*Picea mariana*) et du pin gris (*Pinus banksiana*) dans la forêt boréale, Québec. *Can. J. For. Res.* 22: 474-481.

Sulzer, A.M., M.S. Greenwood, W.H. Livingston and G. Adams. 1993. Early selection of black spruce using physiological and morphological criteria. *Can. J. For. Res.* 23: 657-664.

Syme, P.D. 1995. Insects that attack black spruce in Ontario. Natural Resources Canada, Canadian Forest Service, Sault Ste. Marie, ON. Frontline Tech. Note 77, 4 pp.

Tan, W., and T.J. Blake. 1993. Drought tolerance, abscisic acid and electrolyte leakage in fast- and slow-growing black spruce (*Picea mariana*) progenies. *Physiol. Plant.* 89: 817-823.

Tan, W., and T.J. Blake. 1997. Gas exchange and water relations responses to drought of fast-and slow-growing black spruce families. *Can. J. Bot.* 75: 1700-1706.

Tang, W. and R.J. Newton. 2003. Genetic transformation of conifers and its application in forest biotechnology. *Plant Cell Rep.* 22: 1-15.

Tautorus, T.E., S.M. Attree, L.C. Fowke and D.I. Dunstan. 1990. Somatic embryogenesis from immature and mature zygotic embryos, and embryo regeneration from protoplasts in black spruce (*Picea mariana* Mill.). *Plant Sci.* 67: 115-124.

Thomas, P.A., and R.W. Wein. 1985. Delayed emergence of four conifer species on postfire seedbeds in eastern Canada. *Can. J. For. Res.* 15: 727-729.

Thomson, J., U. Matthes-Sears and R.L. Peterson. 1990. Effects of seed provenance and mycorrhizal fungi on early seedling growth in *Picea mariana*. *Can. J. For. Res.* 20: 1739-1745.

Thompson, R.S., K.H. Anderson and P.J. Bartlein. 1999. Atlas of relations between climatic parameters and distribution of important trees and shrubs in North America. USGS Professional Paper 1650-A (conifers); online at: http://greenwood.cr.usgs.gov/pub/ppapers/p1650-a/ .

Tousignant, D.M., M.R. Villeneuve and S. Mercier. 1995. Effect of tree flowering and crown position on rooting success of cuttings from 9-year-old black spruce of seedling origin. *Can. J. For. Res.* 25: 1058-1063.

Tremblay, L., C. Levasseur and F.M. Tremblay. 1999. Frequency of somaclonal variation in plants of black spruce (*Picea mariana*, Pinaceae) and white spruce (*P. glauca*, Pinaceae) derived from somatic embryogenesis and identification of some factors involved in genetic instability. *Am. J. Bot.* 86: 1373-1381.

Turgeon, J.J. 1990. Clonal variation in susceptibility to damage by insects infesting cones and seeds in black and white spruce seed orchards: preliminary results. Pp. 89-99 *in* R.J. West (ed.), Proc. - Cone and Seed Pest Workshop, St. John's, Newfoundland. 1989. For. Can. Info Rep. N-X-274, 128 pp.

US Geological Survey. 2002. Digital representations of tree species range maps. US Dept. of the Interior Geol. Survey Earth Surface Processes, online at: http://climchange.cr.usgs.gov/data/atlas/little/ .

Van Cleve, K., and J.C. Zasada. 1970. Snow breakage in black spruce and white spruce stands in interior Alaska. *J. For.* 68: 82-83.

Van Cleve, K., L.A. Viereck and C.T. Dyrness. 1982. Dynamics of a black spruce ecosystem in comparison to other forest types: a multi-disciplinary study in interior Alaska. Pp 148-166 *in* R.W. Wein, R.R. Riewe and I.R. Methven,eds. Resources and dynamics of the boreal zone: Proceedings of a conference held at Thunder Bay, ON, Aug., 1982. Assoc. of Can. Univ. for Northern Studies, 544 pp.

Vasiliauskas, S., and H.Y.H. Chen. 2002. How long do trees take to reach breast height after fire in northeastern Ontario? *Can. J. For. Res.* 32: 1889-1892.

Viereck, L.A. 1983. The effects of fire in black spruce ecosystems of Alaska and northern Canada. *In* R.W. Wein and D.A. MacLean. (eds), The Role of Fire in Northern Circumpolar Ecosystems. John Wiley and Sons. 322 pp.

Viereck, L.A., and W.F. Johnston. 1990. *Picea mariana* (Mill.) B.S.P. Black spruce. Pp 227-237 *in* R.M. Burns and B.H. Honkala (eds.), Silvics of North America Vol. 1, Conifers. USDA For. Serv. Agric. Handbook 654, 675 pp.

Villeneuve, M., E.K. Morgenstern and L.P. Sebastian. 1987. Variation patterns and age relationships of wood density in families of jack pine and black spruce. *Can. J. For. Res.* 17: 1219-1222.

Vincent, A.B. 1965. Black spruce: a review of its silvics, ecology and silviculture. Dept. For. Canada Publ. No. 1100, Ottawa, 79 pp.

von Rudloff, E., and M.J. Holst. 1968. Chemostatic studies in the genus *Picea* (Pinaceae). III. The leaf oil of a *Picea glauca* x *mariana* hybrid (Rosendahl spruce). *Can. J. Bot.* 46: 1-4.

Waldron, R.M. 1957. The amount and viability of seed from cones of different ages - black spruce. Can. Dept. For. For. Res. Br. unpubl. report, 7 pp.

Wang, Z.M., and S.E. MacDonald. 1992. Peatland and upland black spruce populations in Alberta, Canada: isozyme variation and seed germination ecology. *Silvae Genet.* 41: 117-122.

Wang, Z.M., and S.E. MacDonald. 1993. Peatland and upland black spruce populations in Alberta: morphology and ecophysiology. *Can. J. For. Res.* 23: 33-40.

Wanner, K.W., B.C. Kostyk and T.R. Meyer. 1995. Tree improvement pest management: Annual report 1994-1995. Ont. For. Res. Instit. Ont. Min. of Nat. Res., 34 pp.

Webb, T. III, P.J. Bartlein and J.E. Kutzbach. 1987. Climate change in eastern North America during the past 18,000 years: comparisons of pollen data with model results. Pp. 447-462 *in* W.F. Ruddiman and J.E. Wright Jr. (eds.), North America and Adjacent Oceans During the Last Deglaciation. Geogr. Soc. Amer., Boulder, Co., The Geology of North America K-3, 501 pp.

Wei, R-P., S.D. Han, N.K. Dhir and F.C. Yeh. 2004. Population variation in growth and 15-year-old shoot elongation along geographic and climatic gradients in black spruce in Alberta. *Can. J. For. Res.* 34: 1691-1702.

Weng, C., and S.T. Jackson. 2000. Species differentiation of North American spruce (*Picea*) based on morphological and anatomical characteristics of needles. *Can. J. Bot.* 78: 1367-1383.

West, R.J. 1986. Seasonal incidence of cone pests of black spruce in Newfoundland. Canadian Forest Service, Newfoundland For. Cen. Info. Rep. N-X-244, 16 pp.

West, R.J. 1989. Cone depredations by the red squirrel in black spruce stands in Newfoundland: implications for commercial cone collection. *Can. J. For. Res.* 19: 1207-1210.

West, R.J., and P. de Groot. 1990. Red squirrels and cone crops: damage and management. Pp. 118-128 *in* R.J. West (ed.), Proc. - Cone and Seed Pest Workshop, St. John's, Newfoundland 1989. For. Can. Info Rep. N-X-274, 128 pp.

White, E.E., R.F. Watkins and D.P. Fowler. 1993. Comparative restriction site maps of chloroplast DNA of *Picea abies, Picea glauca, Picea mariana* and *Picea sitchensis. Can. J. For. Res.* 23: 427-435.

Whitney, R.D. 1989. Root rot damage in naturally regenerated stands of spruce and balsam fir in Ontario. *Can. J. For. Res.* 19: 295-308.

Whitney, R.D. 1994. Minimizing losses to tomentosus root rot in Ontario. Natural Resources Canada, Canadian Forest Service, Sault Ste. Marie, ON. Frontline Tech. Note 82, 4 pp.

Whitney, R.D., and R.L. Fleming. 1995. Disease affects windfall in upland black spruce strip cuts. Natural Resources Canada, Canadian Forest Service, Sault Ste. Marie, ON. Frontline Tech. Note 67: 4 pp.

Wilton, W.C. 1963. Black spruce seedfall immediately following fire. *For. Chron.* 39: 477-478.

Winton, L.L. 1964a. Meiosis and pollen release in white and black spruce and their hybrid. Minnes. For. Notes No. 154, 2 pp.

Winton, L.L. 1964b. Natural polyploidy in juvenile white and black spruce. Minnes. For. Notes No. 144, 2 pp.

Winton, L.L. 1964c. Phenology of normal and forced microsporogenesis in white and black spruce and their F_1 hybrid. Minnes. For. Notes No. 153, 2 pp.

Wright, J.W. 1955. Species crossability in spruce in relation to distribution and taxonomy. *For. Sci.* 1: 319-349.

Yang, K.C., and G. Hazenberg. 1994. Impact of spacing on tracheid length, relative density, and growth rate of juvenile wood and mature wood in *Picea mariana*. *Can. J. For. Res.* 24: 996-1007.

Yeh, F.C., M.A.K. Khalil, Y.A. El-Kassaby and D.C. Trust. 1986. Allozyme variation in *Picea mariana* from Newfoundland: genetic diversity, population structure, and analysis of differentiation. *Can. J. For. Res.* 16: 713-720.

Young J.A., and C.G. Young. 1992. Seeds of Woody Plants of North America. Dioscorides Press. Portland, Oregon, 407pp.

Zasada, J.C., L.A. Viereck and M.J. Foote. 1979. Black spruce seed fall and seedling establishment. Pp 42-50 *in* L.A.Viereck and C.T. Dyrness (tech. eds.), Ecological effects of the Wickersham Dome fire near Fairbanks, Alaska. Pac. Northwest Gen. Tech. Rep. PNW-90.

Zasada, J.C., R.A. Norum, R.M. Van Veldhuizen and C.E. Teutsch. 1983. Artificial regeneration of trees and tall shrubs in experimentally burned upland black spruce / feathermoss stands in Alaska. *Can. J. For. Res.* 13: 903-913.

Zasada, J.C., T.L. Sharik and M. Nygren. 1992. The reproductive process in boreal forest trees. Pp. 85-125 *in* Shugart, H., R. Leemans, and G. Bonan (eds.), A Systems Analysis of the Global Boreal Forest. Cambridge University Press, Cambridge, UK.

Zhang, S.Y., and E.K. Morgenstern. 1995. Genetic variation and inheritance of wood density in black spruce (*Picea mariana*) and its relationship with growth: implications for tree breeding. *Wood Sci. Technol.* 30: 63-75.

Zhang, S.Y., D. Simpson and E.K. Morgenstern. 1996. Variation in the relationship of wood density with growth in 40 black spruce (*Picea mariana*) families grown in New Brunswick. *Wood Fiber Sci.* 28: 91-99.

Zine El Abidine, A., P.Y. Bernier and A.P. Plamondon. 1994a. Water relations parameters of lowland and upland black spruce: seasonal variations and ecotypic differences. *Can. J. For. Res.* 24: 587-593.

Zine El Abidine, A., P.Y. Bernier, J.D. Stewart and A.P. Plamondon. 1994b. Water stress preconditioning of black spruce seedlings from lowland and upland sites. *Can. J. Bot.* 72: 1511-1518.

Zine El Abidine, A., J.D. Stewart, P.Y. Bernier and A.P. Plamondon. 1995. Diurnal and seasonal variations in gas exchange and water relations of lowland and upland black spruce ecotypes. *Can. J. Bot.* 73: 716-722.

Part 2.

Consensus document on safety information on traits

Section 1.
Safety information on transgenic plants expressing *Bacillus thuringiensis* - Derived insect control protein

Summary note

This section summarises the information available on the source of *Bacillus thuringiensis* δ-endotoxin genes, the structure and properties of the toxins they encode, unique mechanisms of action, use in plants, toxicity and exposure data, and assessment methods. Some information on *Bacillus thuringiensis*, the bacterial source of these traits, is included as background and where relevant to the risk assessment of the δ-endotoxins in plants, however this section does not attempt to address the vast amount of information available on the micro-organism. In addition to the scientific literature, which grew substantially over the last few years, this section also contains data submitted by registration applicants for the US-registered plant pesticide products (called plant-incorporated protectants in US pesticide regulations). These studies are required to be performed according to good laboratory practices regulations (US Code of Federal Regulations 40 CFR 160) and have been peer reviewed by USEPA scientists for acceptability for use in an environmental assessment. In the US, data from these studies may be released to the public and are available from the companies on request by other regulatory bodies. Some of these data were submitted for products that are no longer registered; however, the data are still valid to illustrate δ-endotoxin properties. Where it is necessary to illustrate assessments unique to these toxin genes, plant expression data are discussed.

However, the intent of this section is not to address gene transfer or other issues unique to specific plants that have been transformed to express these toxins. Such information is outside the scope of this section. It is intended that this section should be used in conjunction with specific plant species biology consensus documents when a biosafety assessment is made of plants with *Bacillus thuringiensis* δ-endotoxin-mediated insect protection. It was also agreed that this section would not address the issue of insect resistance management, designed to prevent or delay the onset of resistance to specific δ-endotoxins in insects exposed to these transgenic crops.

1. General introduction

Advances in genetic engineering in recent years have led to the development of plants that are resistant to some insects through incorporation and expression of genes encoding delta-endotoxins (δ-endotoxins) from the bacterium *Bacillus thuringiensis* (*B. thuringiensis*). Throughout this paper, the microbial pesticide will be referred to as *Bacillus thuringiensis* whereas the toxins incorporated into the plants will be referred to as δ-endotoxins. Various subspecies of the bacterium, *B. thuringiensis*, are registered as pesticides and are highly regarded as being environmentally-friendly due to their species-specificity (primarily affecting only the pest insects) and their lack of environmental persistence. In addition, δ-endotoxin genes have been inserted into bacteria such as *Pseudomonas fluorescens* (Stone *et al*., 1989) and *Bacillus pumilus* (Selinger *et al*., 1998) for soil insect control, *Clavibacter xyli* for European Corn Borer (ECB) control (Dimock *et al*., 1988), and *Bacillus sphaericus* for mosquito control (Poncet *et al*., 1997), although these have only been used for experimental purposes

in their living form. A Mycogen Corporation product expressing a *B. thuringiensis* δ-endotoxin in *Pseudomonas* was rendered non-viable to address environmental concerns. Four of these products, expressing different δ-endotoxins were registered in 1995 in the United States. A number of plant species, particularly crops such as cotton, corn, potatoes, tobacco, tomato, and sugarcane have been modified to produce δ-endotoxin proteins from B. thuringiensis (Prieto-Samsonov *et al*., 1997; Mendelsohn *et al*., 2003; Romeis *et al*., 2006b).

There are advantages and disadvantages to using transgenic plants containing the δ-endotoxins as compared to the conventional use of microbial *B. thuringiensis* preparations. The control of insects through the expression of δ-endotoxins in the transgenic plant can provide for protection throughout the growing season of the plant. The insecticidal activity need not be short-term, as with conventional Bt preparations which are more rapidly degraded in the environment. Transgenic plants overcome the problem of traditional microbial preparations that may not reach insects that burrow through the soil or those that bore into and remain inside the plant stem or tissue, *e.g.*, the European Corn Borer (ECB) larvae damages the corn stalk from within. Also, microbial preparations have not been as effective as the transgenic cotton/δ-endotoxins product against the Cotton Bollworm (CBW) because the CBW spends most of its time feeding inside the squares (flowers) and bolls (fruit) (Beegle and Yamamoto, 1992). The extended exposure, and relative higher amounts of δ-endotoxins as compared to microbial foliar sprays (Szekacs *et al*., 2005), may lead to the selection of insects that are resistant to one or more of the *B. thuringiensis* δ-endotoxins, thus potentially reducing the usefulness of these *B. thuringiensis* pesticides (Tabashnik *et al*., 1990; Bauer, 1995; Van Rie, 1990b). Tolerant insects have been produced in laboratory studies with purified forms of δ-endotoxins. Various strategies may be employed if deemed necessary to prevent the development of insect resistance in the field (Williams *et al*., 1992; Rajamohan *et al*., 1998; Matten, 1998; Pittendrigh *et al*., 2004; Bates *et al*., 2005).

A major environmental advantage of genetically engineered insect-resistant plants expressing genes encoding δ-endotoxins and of microbial Bt preparations, compared with use of many synthetic chemical insecticides, is the greater specificity of δ-endotoxins to target species. Adverse impacts on non-target insects and other organisms are reduced significantly. In spite of the more targeted specificity, there may still be insects and other non-target organisms potentially affected by the δ-endotoxins, and extended exposure might affect their populations. Another possible disadvantage of genetically engineered insect-resistant plants is a potential for increase in weediness due to δ-endotoxin transgene transfer to populations of wild sexually compatible species. However it should be noted that multiple factors determine the potential for an increase in weediness in wild plant populations, the most important of which is whether the transgene can introgress into related plants. For example, an assessment found that introgression into Australia's 17 native cotton species from the tetraploid cotton crop would not be significant because their native cotton is diploid (AOGTR, 2002). The potential for δ-endotoxin transgene transfer to increase weediness in wild crop relatives has also been studied for sunflower (Snow *et al*., 2003) and for oilseed rape (Halfhill *et al*., 2002, Vacher *et al*., 2004) and is further discussed in paragraph 115 of this document.

1.1. Bacillus thuringiensis *and its uses*

Bacillus thuringiensis is a common bacterium capable of survival in the environment for long periods of time because it produces endospores that are extremely resistant to adverse environmental conditions. Once the spores are in the soil, they do not germinate into vegetative cells unless they are in the presence of a rich nutrient source (Petras and Casida, 1985), e.g. nutrients in the soil or available within organisms that ingest the spores. For example, one tested strain*, B. thuringiensis* subsp. *kurstaki* DMU67R has been shown to persist in the field with no significant reduction in numbers for seven years (Hendriksen and Hansen, 2002), see paragraph 8, below for more details. All members of the genus *Bacillus* are rod-shaped, Gram positive cells that produce not more than one endospore per cell. Cells have peritrichous flagella surrounding them and are aerobic or facultatively anaerobic. Sporulation is not repressed by

exposure to air (Claus and Berkeley, 1986). The species *B. thuringiensis* is characterised by the production of one or more protein parasporal crystals in parallel with spore formation. The parasporal crystals consist mainly of insecticidal δ-endotoxins with some scaffolding proteins and Cyt toxins. The δ-endotoxins in the crystals are usually inactive protoxins, which are converted by enzymatic action within the environment of the larval gut to active toxins (Claus and Berkeley, 1986). These toxins, in addition to other toxins produced by some isolates of *B. thuringiensis*, account for the insecticidal activity of the commercialised products to lepidopteran, dipteran, and coleopteran insects. The microbial products often show some additional activity, compared to δ-endotoxins alone, by expressing other factors while reproducing within the insects.

Naturally-occurring isolates of *B. thuringiensis* have been used for insect control for decades. The first description of a *Bacillus thuringiensis* bacterium was in 1901 by the Japanese microbiologist S. Ishiwata who isolated it from diseased silkworm larvae (Ishiwata, 1901). Ishiwata named the bacillus Sottokin. A decade later, a German microbiologist, E. Berliner, isolated a similar organism from a diseased granary population of *Ephestia kuehniella* larvae from Thuringia, Germany (Berliner, 1911, 1915; also cited in Beegle and Yamamoto, 1992). Berliner named the bacterium *Bacillus thuringiensis*, and because Ishiwata did not formally describe the organism he found, Berliner is credited with naming it. The first commercial *B. thuringiensis* product was produced in France in 1938 (Kumar *et al*., 1996). An isolate was first registered as an insecticide in the United States in 1961. Microbial preparations of various isolates of *B. thuringiensis* are used on a wide variety of grain, forage, fruit, vegetable, tuber, and fibre crops, and tobacco. In addition, they are used for control of forest pests, particularly gypsy and tussock moth species, and also for control of mosquitoes and blackflies.

When applied as a microbial insecticide, *B. thuringiensis* toxins have a relatively short persistence of 1 - 4 days on plants due to degradation from UV light exposure, however, a study of a Bt forest spray showed continued toxicity toward lepidopterans for at least 30 days following the spray (Johnson *et al*., 1995). The *B. thuringiensis* spores persist in the environment for extended periods, and have been isolated world-wide from soil (Martin and Travers, 1989; Bernhard *et al*., 1997; Ejiofor and Johnson, 2002), and from plant surfaces (Smith and Couche, 1991). Typically, *B. thuringiensis* is not naturally found in high numbers, except in previously treated soils, but it is not rare. Significant numbers of various strains of *B. thuringiensis* have been found in many different kinds of soils in Denmark including areas where commercial products have not been used (Hendriksen and Hansen, 2004). Delucca *et al*. (1981) found it in 17% of the soils they tested from 12 US states and reported it was found in a wide variety of soils: cultivated soils, a rocky soil, and in virgin (not previously treated) forests. Spores of *B. thuringiensis* can maintain their presence in the environment by germinating and replicating to high numbers in suitable hosts which are not harmed by their presence. Many animals have been shown to excrete *B. thuringiensis* in their faeces. These include voles (Swiecicka and De Vos, 2003), Japanese deer (Ohba and Lee, 2003), 14 species of wild mammals in Korea (Lee *et al.*, 2003) and 11% of rodents and 17% of insectivore mammals examined (Swiecicka *et al*., 2002). These mammals may also include humans since *B. thuringiensis* was found to be a common part of the microbial flora in sewage plant sludge (Mizuki *et al*., 2001). The same process has also been observed in soil inhabiting invertebrates since *B. thuringiensis* was shown to germinate in three species of earthworm and one tipulid larvae without harming them (Hendriksen and Hansen, 2002). Thus, *B. thuringiensis* spores and toxins are an integral part of the environment.

The microbial *B. thuringiensis* products, which contain differing numbers of δ-endotoxins, may be toxic to a number of different species of insects from different genera, see Section 1C for more detail and the tables in appendixes 2.2 and 3.2 of Glare and O'Callaghan (2000) for extensive lists of insects resistant to different strains of *B. thuringiensis*. Many pest insects are resistant to the *B. thuringiensis* δ-endotoxins. For example, the European cockchafers are significant pest insects but have developed protective proteolytic midgut enzymes that protect them against Cry8C which kills closely-related scarab insects (Wagner *et al*., 2002). The results of various studies on the susceptibility of pest insects to

Bt kurstaki spray were analysed by Schmitz *et al*. (2003). Among those groups for which sufficient data were available, the Geometridae appeared to be the most susceptible family. In contrast, the Noctuidae are relatively resistant to Bt spray. Overall the literature confirms the lepidopteran-specific toxicity of commercial *Bt kurstaki* toxins (Schmitz *et al*., 2003).

1.2. Bacillus thuringiensis *toxins*

Most of the δ-endotoxins from *B. thuringiensis* are contained in the parasporal crystal inclusions that are synthesised adjacent to the endospore during sporulation. The parasporal crystal inclusions consist of different insecticidal crystal proteins, each of which is coded for by a single gene. Depending on the composition of the insecticidal proteins, the crystals can occur in a number of shapes, such as bipyramidal, cuboidal, flat rhomboid, or a composite with two crystal types. The genes that code for the insecticidal crystal proteins are usually located on plasmids, which are autonomously replicating circular pieces of extrachromosomal DNA that may be transferred by conjugation between various serovars of *B. thuringiensis* and related bacterial species such as *Bacillus cereus* and *Bacillus subtilis* (Klier *et al*., 1983, Battisti *et al*., 1985, and Ruhfel *et al*., 1984.) The *B. thuringiensis* plasmids are relatively large and may contain one quarter of the genetic coding capacity of the bacterial chromosome (Carlton and Gonzalez, 1985). Schnepf *et al*. (1998) noted that there is considerable evidence that *B. thuringiensis* and *B. cereus* should be considered a single species. A genetic analysis of many isolates of *B. thuringiensis, B. cereus,* and *Bacillus anthracis* has found extensive genetic diversity among *B. thuringiensis,* and *B. cereus* environmental isolates with no clear distinction between the two species. However, the *B. anthracis* strains were more closely related to each other (Ticknor *et al*., 2001). Strains of *B. anthracis* also exhibit much less diversity (Keim *et al*., 1997). The *B. thuringiensis* strains used for insecticides cluster separately from the closely grouped *B. anthracis* strains, except that the one H34 strain previously identified as producing a δ-endotoxin, and had been reported to be pathogenic to mice, (Hernandez *et al*., 2000) occurred in the branch that contained all the *B. anthracis* strains (Hill *et al*., 2004).

The δ-endotoxins include another *B. thuringiensis* toxin type, which has cytolytic activity against a number of invertebrate and vertebrate cells *in vitro*. These "Cyt" toxins have been shown to have specific activity on dipteran insects *via* a mode of action similar to the Cry toxins. The interaction of Cyt toxins with Cry toxins is complex because in some cases the toxicity of a given Cry/Cyt toxin combination is synergistic and in others antagonism is found, *e.g.* between Cry1Ac1 and Cyt1A1 both *in vitro* and *in vivo* toxicity to *Trichoplusia ni* (Del Rincon-Castro *et al*., 1999).

Recently binary toxins from Bt have been assigned a Cry designation, though they have little detectable homology to traditional Cry toxins. Dow AgroSciences LLC and Pioneer Hi-Bred International recently registered a binary toxin, Cry34Ab1/Cry35Ab1, from the Mycogen Corporation *B. thuringiensis* toxin library (USEPA, 2005). Monsanto also holds a patent on a binary toxin.

In addition to the δ-endotoxins, other toxins may be produced by various isolates of *B. thuringiensis.* One such proteinaceous toxin class from *Bacillus* isolates is Vegetative insecticidal protein (Vip) 3A (Estruch *et al*., 1996) which has broad toxicity against lepidopteran species (C.Yu *et al*., 1997). Genetically engineered products expressing Vip3A are being evaluated in cotton and maize plants. Although it has similar properties to the δ-endotoxins, the Vip3A toxin has not been classified as a δ-endotoxin and will not be addressed in this document. Some isolates of *B. thuringiensis* produce a class of closely related adenine-nucleotide analogue insecticidal molecules called beta-exotoxin, (Hernandez *et al*., 2001). The common name for the beta-exotoxins is thuringiensin. These heat-labile toxins may be responsible for the toxicity of some isolates of *B. thuringiensis* to non-target organisms including mice, some aquatic insects, and fish (Beegle and Yamamoto, 1992). Beta-exotoxin and the other *Bacillus* toxins may contribute to the insecticidal toxicity of the bacterium to lepidopteran, dipteran, and coleopteran insects (Crickmore *et al*., 2005). Beta-exotoxin is known to be toxic to humans and almost all other forms of life and its presence is prohibited in *B. thuringiensis* microbial products. Engineering

of plants to contain and express only the genes for δ-endotoxins avoids the problem of assessing the risks posed by these other toxins that may be produced in microbial preparations.

1.3. Susceptible insects

Various isolates of *B. thuringiensis* have been reported to have pesticidal activity against insect species, primarily lepidopteran, coleopteran and dipteran species, and some non-insect species, for example nematodes, flatworms, protozoa (Feitelson *et al*., 1992; Griffitts *et al*., 2001; Kondo *et al*., 1992), and also mites (Arachnida, Acarinae) (Feitelson *et al*., 1992). However, some of this activity observed for bacterial isolates may be due to *Bacillus* toxins other than δ-endotoxins. There are many different δ-endotoxins and they have vastly different specificities against different insects. This great diversity is likely to have developed through sequence divergence and subsequent swapping of domains within the toxin molecules (de Maagd *et al*., 2001). Many different δ-endotoxins have been tested for activity against various insects both at the strain and individual toxin level. Most of the insects reported as susceptible to δ-endotoxins are Lepidoptera, but many δ-endotoxins are active against Diptera (*e.g.* Cry4, 10, 11, 19, and 25) and Coleoptera (*e.g.*, Cry3 and 8). Among the Lepidoptera, there is variation in susceptibility to various δ-endotoxins. In a survey of 42 species of non-target lepidopterans, 13 species were rated as very sensitive to a commercial product, Foray 48B (containing genes encoding, but not necessarily expressing, the Cry1Aa1, Cry1Ab1, Cry1Ac1, Cry2Aa1, and Cry2Ab1 proteins), and 11 species were rated as insensitive (Peacock *et al*., 1998). Some δ-endotoxins do not appear to directly affect any tested pest species. Lambert *et al*. (1992) reported that a Cry7Aa did not affect various lepidopteran larvae and was only weakly active against Coleoptera although *in vitro* pre-treatment with trypsin increased its activity against Coleoptera. Some representative δ-endotoxins have been analyzed to the extent that host range and toxicity functions have been attributed to different domains of the protein molecule. The natural *B. thuringiensis* isolates generally produce more than one δ-endotoxin. Many experiments, both *in vitro* and *in vivo,* suggest synergistic interactions between two or more δ-endotoxins (Schnepf *et al*., 1998). On the other hand, antagonism was also observed between Cry1Aa and Cry1Ab in the gypsy moth (Tabashnik, 1992).

Caution should be used when attempting to extrapolate the susceptible species to an individual δ-endotoxin as expressed in plants from tests using microbial forms of *B. thuringiensis*. As noted before, most strains of *B. thuringiensis* express multiple toxins, which often may interact with each other. In some cases, δ-endotoxins combined with spores can exhibit toxicity even though neither the spores nor the toxins, by themselves, are especially toxic to the test insect (Liu *et al*., 1998). Frequently the δ-endotoxin may be expressed in a plant in a truncated form, and it has been hypothesised this situation could increase the host range of the δ-endotoxin to additional insects. It should be noted, however, that other factors contribute to the selectivity (see paragraph 30 of this document). Clearly, protocols screening for insect susceptibility need to ensure that test insects are exposed to Cry toxins that are the same or equivalent to Cry toxins that are expressed in the insect-resistant plants in question; for example use of Cry toxins isolated from *E.coli* strains, grown under contained conditions, that have been genetically engineered to express the relevant δ-endotoxin.

2. Nomenclature/Classification of toxins and genes

Unlike some systems where genes were named without knowledge of the gene products, δ-endotoxins and the genes which produce them rely on the same nomenclature. However, standard nomenclature for genes requires that the gene name be italicised in lower case (*e.g. cry1A*), whereas, the δ-endotoxin protein product produced by that gene is designated in regular font with an initial capital letter (*e.g.* Cry1A).

Hofte and Whiteley (1989) proposed a classification scheme that related similar toxin gene sequences to the activity against susceptible insects. From a total of 52 genes, Hofte and Whiteley designated

14 distinct gene types and sorted them into four major classes based on their insect specificity. The four major gene classes consisted of *cryI* - Lepidoptera; *cryII* - Lepidoptera and Diptera; *cryIII* - Coleoptera; and *cryIV* - Diptera. More recently, two new classes were proposed, *cryV* and *cryVI*, (Feitelson *et al*., 1992) based on additional analysis of the toxin domains of 29 distinct toxin proteins. This classification scheme, however, is no longer adequate to identify the many new varieties of δ-endotoxin genes, in that some of the newly-analyzed genes show a high degree of DNA homology to known genes, but possess different insecticidal activities. Therefore, in 1993, a δ-endotoxin nomenclature committee was established to revise the classification of δ-endotoxins.

Crickmore *et al.* (1998) introduced a systematic nomenclature based on the similarity between amino acid sequences of full-length gene products, rather than their biological properties. The scheme, which arranges the genes according to possible evolutionary relationships, is based on a phylogenetic tree calculated with computer programs that are in the public domain. The *cry* genes designated by Hofte and Whiteley (1989) have been retained, although the Roman numerals have been replaced by Arabic numbers (*cryII* is now *cry2*), still followed by uppercase and lower case letters. This new nomenclature scheme defines the degree of homology that needs to be shared to have the same Arabic number (≥ 45%), the same uppercase letter (≥ 75%), and the same lowercase letter without parentheses (≥ 95%). A fourth ("quaternary") ranking is also given for gene products that differ in amino acid sequence but whose genes have more than 95% homology. For example, the gene designated by the Hofte and Whiteley (1989) classification scheme as *cryIA(c)* by Von Tersch *et al.* (1991) is currently designated as *cry1Ac2*. Note that a different quaternary ranking is assigned for each new Cry toxin submission, so that some of the toxins that have identical nomenclature except for different quaternary rankings may actually be identical. Although it may be argued that this system does not exactly meet some of the standards set for protein nomenclature, it has the advantage that the genes classified under the earlier system, for the most part, do not need a major change in their name, and there is no need to make changes in the vast existing literature. A few *cry* genes have been reassigned under the new system (Table 1). A *Bacillus thuringiensis cry* Gene Nomenclature Committee is now part of the Bacillus Genetic Stock Center. A current list of δ-endotoxin genes can be found on the Internet at http://www.lifesci.sussex.ac.uk/Home/Neil_Crickmore/Bt/ (Crickmore *et al*., 2005).

Under the new classification system, there are 49 major classes of different *cry* genes, *cry1* through *cry49*, and two major classes of *cyt* genes, *cyt1* and *cyt2*. At the end of the year 2005, there were a total of 314 different *cry* genes (including 26 binary *cry* genes) and 24 *cyt* genes. In recent years, about 20 newly classified genes are generally added each year. Use of this nomenclature will greatly facilitate international harmonisation of δ-endotoxin regulatory assessment. Wherever possible, this nomenclature has been used in this document, however, in some cases, the older nomenclature is used when it was referenced as such in citations.

Table 1. Some of the renamed δ -endotoxin genes (Old *vs*. new nomenclature)

Old	*cryIG*	*cryIIIC*	*cryIIID*	*cryIVC*	*cryIVD*	*cytA*	*cytB*
New	*cry9A*	*cry7Aa*	*cry3C*	*cry10A*	*cry11A*	*cyt1A*	*cyt2A*

Source: Crickmore *et al*., 1998

3. Characterisation of the δ-endotoxins

3.1. Protoxins

The parasporal crystalline inclusion bodies formed within the *B. thuringiensis* cells adjacent to the endospore during sporulation are protoxins which are composed of precursors of the active δ-endotoxins and DNA (Clarimont *et al*., 1998). For the three conventional three-domain toxins, *e.g*. Cry1, Cry2 and Cry3, the C-terminal half of these inactive protoxins are enzymatically cleaved within the midgut of susceptible insect larvae by trypsin-like proteases to the active toxin, which consists of the N-terminal portion of the molecule (Federici, 1993; Rukmini *et al*., 1999).

The lepidopteran-specific Cry1 protoxins are 130 – 140 kDa in size and except for Cry1I accumulate as bipyramidal crystalline inclusion bodies. There are a large number of different *cry1* gene sequences (Hofte and Whitely, 1989; Crickmore *et al*., 1998; Crickmore *et al*., 2005). The Cry1A through Cry1G protoxins are in the range of 1100-1200 amino acids and the active portion of the protoxin molecule is a 60 - 70 kDa fragment localised in the N-terminal half of the protoxin for the Cry1A and Cry1C proteins. For many of the Cry toxins the proteolytic cleavage site is not known experimentally, however it can be inferred from homology modelling approaches. The bipyramidal Cry4A and Cry4B protoxins, which are approximately 130 kDa, also consist of approximately 1100-1200 amino acids (Knowles, 1994; Kumar *et al*., 1996).

The Cry2, Cry3, and Cry11A protoxins are smaller molecules of approximately 70 kDa which are similar to the N-terminal portion of the larger protoxins (as reviewed by Bauer, 1995). Crystals formed from the Cry2 proteins are cuboid, the Cry3 are rhomboid, and the Cry10A and Cry11A are bar-shaped (Knowles, 1994). These smaller protoxins still require enzymatic processing involving removal of amino acids from the N terminus to form the active toxins (Bauer, 1995). Cry11A can be processed differently than the other Cry toxins in some insect species. It is cleaved into two fragments of 30 and 35 kDa. The Cyt protoxins are smaller molecules of 29 kDa and have an amorphous crystal shape in the absence of Cry toxins (Knowles, 1994; Li, 1996). After solubilisation the toxin is present as a dimer and can be cleaved by proteinase K to uncover the active sites *in vitro* (Koni and Ellar, 1994; Li, 1996).

The variation in specificity of δ-endotoxins to different species of insects may be, in some cases, due to the presence of different proteolytic enzymes. Cry1Ac is very active against *Mamestra brassicae* (Cabbage moth), but has little effect on *Pieris brassicae* (Cabbage white butterfly); two insects that are not closely related, being from different families. Extended proteolysis using proteases from each insect resulted in insoluble products, but with different molecular sizes resulting from differential processing (Lightwood *et al*., 2000).

3.2. Truncated active toxins

Truncated active toxins are the N-terminal portion of the protoxin molecules obtained after enzymatic cleavage within the insect midgut of the larger Cry toxins. It is this portion of the protoxin which then binds to receptors and ultimately results in lethality due to membrane disruption. The smaller 70 kDa Cry2, Cry3, and Cry11 are sometimes considered truncated forms of the N-terminal portion of the larger Cry toxins, although some processing still occurs on these smaller molecules (Knowles, 1994). The 29 kDa Cyt protoxins are dimers that are cleaved into active monomers (Koni and Ellar, 1994; Li, 1996).

3.3. Structure of toxins

The three-dimensional structures of the coleopteran specific Cry3A (Li *et al*., 1991) and the mosquitocidal Cyt2A δ-endotoxins (Li *et al*., 1996) have been published. The structure of the lepidopteran specific Cry1Aa has also been determined and is similar to the Cry3A structure

(Grochulski *et al*., 1995). In addition, the structures for Cry2Aa (Morse, at al., 2001) and Cry3Bb1 (Galitsky *et al*., 2001) have recently been published. Based on their sequence similarity, many other Cry δ-endotoxins are thought to have a similar three-dimensional structure. The first 285 amino acids are a bundle of seven amphipathic helices. Six of these helices occur in a circle surrounding helix five in the centre of the Cry3A molecule. These helices are known as domain I. Domain II consists of amino acid residues 286-500 which form three antiparallel β-sheets. Domain III is the rest of the amino acids in β-sheets arranged like a sandwich (as reviewed by Kumar *et al*., 1996).

The Cyt protoxins have just a single domain in which two outer layers of α-helices surround a five stranded β-sheet. The protoxin is actually a dimer of two of these molecular domains associated at the N-terminus strands (Li, 1996).

3.4. Prevalence of the δ-endotoxins in micro-organisms

It is common for microbial *B. thuringiensis* strains to express more than one δ-endotoxin, yet the same δ-endotoxins may appear in many different isolates. Two published studies, in particular, examined the prevalence of certain δ-endotoxins. The distribution of δ-endotoxin genes in 58 new isolates showed 57% had *cry1C*, 45% had *cry1A(b)*, and 34% had *cry2A* genes (Kim *et al*., 1998). Another study of 223 isolates looked at three families of δ-endotoxin genes (Ferrandis *et al*., 1999). They found *cry5* δ-endotoxin genes in 66%, *cry1* in 54% and *cry2* in 42% of the isolates. A specific analysis of the isolates possessing *cry1* genes showed 62% had *cry1A(c)*, 49% had *cry1A(a)*, 43% had *cry1D*, 35% had *cry1C*, and 34% had *cry1A(b)* δ-endotoxin genes (Ferrandis *et al*., 1999).

The microbial *B. thuringiensis* strains are generally divided taxonomically into serovars based on differences in the antigens in their flagella, *e.g. B. thuringiensis* ser. *kurstaki*, or *B. thuringiensis* ser. *israelensis*. In some cases biochemical and morphological criteria are used to further distinguish the serovars. However, since the δ-endotoxin genes are primarily carried on large plasmids with some mobility, the subspecies designations do not definitively allow predictions of their specific *cry* gene content. One analysis of Bt isolates reported that there is no apparent relationship between δ-endotoxin gene content and serotype of the micro-organism (Ferrandis, 1999). Bacterial species other than *B. thuringiensis* have been shown to produce δ-endotoxins. In 1990, crystalline δ-endotoxin-like proteins were first reported in *Clostridium bifermentans,* serovar *malaysia* (de Barjac *et al*., 1990) and subsequently found in 80% of 12 *C. bifermentans* subspecies and 8% of 13 other *Clostridium* strains and 13 *B. thuringiensis* isolates tested (Barloy *et al*., 1998). A Cry2Aa δ-like endotoxin, termed Cry18Aa, has been detected in *Bacillus popilliae*, which is a US registered microbial pesticide product for control of the Japanese beetle (Zhang *et al*., 1997).

4. Mechanism of action

An immense amount of research on representative δ-endotoxins has been devoted to understanding the mode of action on susceptible insects. In general, following ingestion, the crystalline inclusions are dissolved and then converted to active toxins by insect proteases. The active toxins bind to specific receptor sites and produce pores in the insect gut which results in loss of homeostasis and septicemia, which are lethal to the insect (Broderick *et al*., 2006). In addition, there may be other less-characterised insect control functions of these toxins such as avoidance of the toxins and feeding paralysis prior to completion of the full lethal pore-formation process (Aronson and Shai, 2001). In many cases, larvae become less susceptible to δ-endotoxins as they age due to fewer binding sites in the older larvae (Gilliland *et al*., 2002).

4.1. Ingestion and solubilisation

When the non-toxic *B. thuringiensis* crystalline inclusions are ingested by a susceptible lepidopteran insect larva, they dissolve in the high pH (>9.5) environment of the larval midgut, releasing one or more δ-endotoxins. However, many coleopterans have a neutral pH midgut, yet solubilisation of the coleopteran-specific toxins occurs (Koller *et al*., 1992). Solubilisation may occur due to initial proteolysis of Cry3A, which renders the toxin soluble at neutral pH allowing it to impart activity against coleopterans (Carroll *et al*., 1997). Combinations of Cry proteins in inclusion bodies may also facilitate the solubility over that of crystals with only one Cry protein (Aronson, 1995). These proteins are protoxins that are converted enzymatically in the insect midgut by proteases into smaller active toxins which are resistant to further protease digestion. These active toxins bind to unique receptor sites on the epithelium cells in the midgut of susceptible insects. The proteolytic susceptibility of various Cry1A protoxins appears to be sensitive to DNA associated with the N-terminal end of the protoxin in the crystals (Clairmont *et al*., 1998).

The ingestion of plant material containing the δ-endotoxin, in the form of the protoxin or as an active truncated toxin, has been shown by many studies to control the same target hosts as does ingestion of *B. thuringiensis* crystalline inclusions containing the δ-endotoxins (Mycogen and Novartis, 1995a, 1995b; Plant Genetic Systems, 1998c; Mycogen and Pioneer, 2001a, 2001b, 2001c, 2005a; Monsanto, 2002b, 2002f). It has been proposed that the solubilisation and proteolysis phase can contribute to the selectivity of action towards susceptible insects, and that if the δ-endotoxin expressed in the plant is the truncated active form, a wider range of hosts may be affected (Stotzky, 2002, Hilbeck, 2002). However, there is no evidence to support the hypothesis that protease activated or truncated toxins alter the host range of non-target insects (Mycogen and Novartis, 1995a, 1995c; Plant Genetic Systems, 1998c; Mycogen and Pioneer, 2001a, 2001b, 2001c, 2005a; Monsanto, 2002b, 2002d; Evans, 2002). Additional studies will be required to shed more light on these issues (Evans 2002).

4.2. Binding to receptors

For the classic three-domain Cry toxins, *e.g.*, Cry1A and Cry2A, binding of the active Cry toxin molecules to a receptor in the brush border membrane of the epithelial cells in the midgut microvillae of the target insect is an essential process in achieving toxicity (Hoffman *et al*., 1988), although additional post-binding processes, including membrane insertion and septicaemia, are required for insect lethality (Broderick *et al*., 2006). The specificity of the Bt δ-endotoxins is a primary result of their ability to bind to specific receptor sites in the membrane. However, it has been shown that many toxins are capable of binding to more than one receptor (Van Rie *et al*., 1989; Van Rie *et al*., 1990a; Denolf *et al*., 1993; Estada and Ferré, 1994; Escriche *et al*., 1994), one receptor can bind more than one toxin (Escriche *el al*., 1997), and different Cry toxins can compete for the same binding sites (Hua *et al*., 2001; Estela *et al*., 2004; Li *et al*., 2004). The amino acid sequence of the receptor binding domain of the δ-endotoxin molecule is thought to be a predictor of host specificity (as reviewed by Bauer, 1995). The binding domain of the δ-endotoxin molecule is apparently the most variable portion of the active toxin molecule (Hofte and Whitely, 1989). In some cases binding of the active toxin molecule correlates directly with toxicity (Luo *et al*., 1997; Jurat-Fuentes *et al*., 2000), but binding has not always exhibited a correlation with toxicity *in vivo* (Van Rie *et al*., 1990a; Gould *et al*., 1992; Escriche *et al*., 1994). The association of binding with potency is further supported in that some Cry1 toxins showed correlation with potency in some but not all cases studied (Gilliland *et al*., 2002). Lee *et al*. (1999) hypothesised that cases where there is no correlation may be due to non-functional receptors, or alternatively, to initial binding to more than one receptor.

The high affinity binding of the active toxin molecule to the specific receptor within the insect midgut is thought to occur by interaction of the loops of domain II, the portion of the active toxin with three antiparallel β-sheets (as reviewed by Knowles, 1994). A revised model for the mechanism of action

has been proposed based on studies conducted using site-directed mutagenesis of the Cry toxins. Dean *et al*. (1996) and Aronson and Shai (2001) suggest that domain III is also involved in the binding of the active toxin molecules to the receptors for many insects. For example, de Maagd *et al*. (2000) showed that domain III was essential for toxicity to *Spodoptera exigua* by producing *Spodoptera* activity in several Cry1 toxins by transferring an active domain III from Cry1Ca into them. However, in the specific case of the diamondback moth (*Plutella xylostella*), domain III was found to have a minimal effect on toxicity and binding (Ballester *et al*., 1999).

Many putative receptors have been identified for various δ-endotoxins in a number of different insects. The emerging picture is complex. The same toxin may bind to different receptors and different toxins may bind to the same receptors. For example, Cry1C is reported to bind to both a 40 kDa protein (Kwa *et al*., 1998) and a 106 kDa aminopeptidase-N glycoprotein (Luo *et al*., 1996). Wang and McCarthy (1997) identified seven Cry1C binding proteins (137, 120, 115, 68, 63, and 45 kDa). Cry1Ab and Cry1Ac compete for the same binding site in the striped stem borer (*Chilo suppressalis*) and the yellow stem borer (*Scirpophagus incertulis*) (Alcantara *et al*., 2004). Cry1Ac domain III mutants could no longer bind to aminopeptidase-N, however, some toxicity remained to *Manduca sexta* indicating the presence of alternative receptors (Jenkins *et al*., 1999).

Aminopeptidase-N proteins, belonging to a family of zinc-dependent metallopeptidases, have been shown to function as receptors for many δ-endotoxins proteins. Cry1Aa was shown to bind to a highly conserved region of the aminopeptidase-N family of proteins (Nakanishi *et al*., 1999). A detailed analysis of the *Tenebrio molitor* midgut aminopeptidase revealed some common features with mammalian aminopeptidase-N, but it differed in details of substrate binding and in catalytic residues (Cristofoletti and Terra, 2000). Luo *et al*. (1997) have found that a specific 170 kDa aminopeptidase-N from *Heliothis virescens* would bind Cry1Aa, Cry1Ab, and Cry1Ac, but not Cry1C or Cry1E. A 120 kDa aminopeptidase-N from brush border membrane vesicles of a tortricid moth was shown to bind both Cry1Ac and Cry1Ba (Simpson and Newcomb, 2000). Two amino acid differences in aminopeptidase-like proteins were sufficient to make an Indianmeal moth (*Plodia interpunctella*) resistant to Cry1Aa (Zhu *et al*., 2000). The receptor for the Cry1A(c) toxin in the lepidopteran *Manduca sexta* was identified as a 120 kDa aminopeptidase-N (Knight *et al*., 1994). Two δ-endotoxins, Cry1Ac and Cry1Fa, bind to several aminopeptidase-N's (110, 120, and 170 kDa.) from *Heliothis virescens* (Banks *et al*., 2001). A thorough investigation of one system, Cry1Ac binding to *Lymantria dispar* aminopeptidase-N, suggested that an initial recognition of the aminopeptidase occurs by a region in domain III of Cry1Ac and a subsequent tighter binding occurs *via* a region in domain II (Jenkins *et al*., 2000). However, the study using Cry1C, Cry1E, and Cry1Ab in *Plutella xylostella* showed that binding specificity was due to domain II with no detectable involvement with domain III (Ballester *et al*., 1999).

The aminopeptidase-N family of neutral zinc-dependent metallopeptidases has been well studied. They are classified in an M1 family, which, in turn, is part of a superfamily of 36 families. Currently there are two unique recognised classes, bacterial aminopeptidase-N and mammalian aminopeptidase-N. There have been a number of research efforts focusing on cloning and sequencing the genes coding for specific insect aminopeptidase-N δ-endotoxin receptors (Knight *et al*., 1995; Denolf *et al*., 1997; Hua *et al*, 1998; Garner *et al*., 1999; Yaoi *et al*., 1999; Emmerling *et al*., 2001). These aminopeptidases play an important part in insect digestion of proteins, cleaving single amino acids from the N-terminus end (Ortega *et al*., 1996). The sequencing information now available has allowed for the conclusion that the insect aminopeptidase-N's are a unique, distinct, group among the aminopeptidases (differing from the bacterial and mammalian aminopeptidases) and, among themselves, are quite diverse, falling into at least three distinct groups of midgut aminopeptidases (Gardner *et al*., 1999; Emmerling *et al*., 2001).

Carbohydrates may be involved in aminopeptidase-N binding, resulting in further specificity of δ-endotoxins binding to insect midgut cells. A Cry1Ac domain III mutant δ-endotoxin was developed which caused reduced binding to *Manduca sexta*. This mutant Cry1Ac δ-endotoxin was not inhibited by

the carbohydrate, N-acetylgalactosamine, which did inhibit binding of the wild-type Cry1Ac, indicating that this carbohydrate in the aminopeptidase-N from *Manduca sexta* is involved in the mechanism of toxin binding (Burton *et al*., 1999). This is not always the case because a 100 kDa aminopeptidase-N from *Heliothis virescens*, which was bound by Cry1Ac and Cry1Fa, did not contain the N-acetylgalactosamine carbohydrate (Banks *et al*., 2001). Further carbohydrate involvement with binding was shown in a system using plant-pathogenic nematodes that were susceptible to Cry5B and Cry14A (Griffitts *et al*., 2001). Nematodes with mutation-inactivated β-1,3 galactosyltransferase genes were resistant to the δ-endotoxins. The enzyme galactosyltransferase catalyzes the transfer of galactose to glycoproteins and glycolipids. Further research strongly suggests that the binding receptor for Cry5B in susceptible nematodes is a carbohydrate (Huffman *et al*., 2004). Recent studies suggest that nematicidal and insecticidal three-domain Bt toxins use invertebrate glycolipids as host cell receptors (Griffitts *et al*., 2005).

Another class of δ-endotoxin receptors has been shown to be related to the superfamily of cadherin proteins. Cadherins are calcium-dependent proteins that are generally known for their cell to cell adhesion properties. A cadherin-like 175 kDa glycoprotein (BtR175) from *Bombyx mori* was shown to be a receptor for Cry1Aa (Nagamatsu *et al*., 1998). Addition of the gene for BtR175 to Cry1Aa-resistant *Spodoptera fugiperda* Sf9 cells *in vitro* rendered them susceptible to the toxin (Nagamatsu *et al*., 1999). Another cadherin-like 210 kDa glycoprotein was found in *Manduca sexta* that binds to the Cry1Ab δ-endotoxin (Vadlamudi *et al*., 1995; Francis and Bulla, 1997). In addition, *Heliothis virescens* was shown to have a cadherin-like receptor protein for Cry1Ac (Gahan *et al*., 2001).

The cadherin superfamily consists of at least six subfamilies. The invertebrate cadherins occupy an isolated position in the superfamily (Nollet *et al*., 2000). The uniqueness of these insect binding proteins gives further insight into the observed lack of mammalian toxicity for these Cry toxins.

Knowledge of the receptor binding process of the Cyt δ-endotoxins toxins is not as extensive as with the Cry toxins. Based on *in vitro* experiments with artificial membranes, it was originally thought that the cytolytic toxins, which are capable of lysing a wide range of invertebrate and vertebrate cells including mammalian erythrocytes (Hofte and Whiteley, 1989), inserted directly into the insect midgut membrane without binding to a specific receptor. However, more recent data on mosquitoes has suggested that the Cyt toxins, particularly the toxin Cyt1A, bind to a specific region in the midgut (Ravoahangimalala *et al*., 1993; Ravoahangimalala and Charles, 1995). The binding process appears to be more closely associated with the membrane disruption process than for the Cry toxins (Li *et al*., 1996; Luo *et al*., 1997; Du *et al*., 1999).

4.3. Pore formation and bacterial septicemia

Both binding and pore formation are necessary for optimum activity against insects. It has been shown that binding of the toxin alone is not enough to cause toxicity. Two proteins in the gut membranes of *Tenebrio molitor* larvae (137 and 107 kDa, respectively) were shown to bind Cry1Aa, however the insect is resistant to that toxin (Nagamatsu *et al*., 1998). Escriche *et al*. (1998) showed that Cry1Ab would bind to *Spodoptera littoralis* midgut receptors, but would not produce pores and *in vivo* assays show that Cry1Ab is only marginally active against *S. littoralis*. Following the binding of active δ-endotoxins to specific receptors on the brush border membrane in the insect midgut, the toxins insert into the membrane. The toxins, both Cry and Cyt, intercalate irreversibly into the membrane.

After insertion of the Cry δ-endotoxin, several receptor-toxin complexes then form aggregates that form pores in the membrane (Walters *et al*., 1993; Knowles, 1994; Soberon *et al*., 2000). The pores formed in the plasma membrane disrupt the osmotic balance within the cells which causes the cells to swell and burst. At this point, the insects stop feeding. Domain I in Cry proteins was shown to be a pore forming domain (Walters *et al*., 1993; VonTersch *et al*., 1994). The left-handed supercoil of domain I, made up of α-helices, is "clearly equipped for membrane insertion" (Li, 1996). Furthermore,

the α-helices of this domain, while sharing no amino acid similarity, resemble domains in diphtheria toxin and colicin A that also form pores in membranes. Research by Schwartz *et al*. (1997) suggests an interaction with domain III may also have some effect on the pore formation in membranes. Investigations are proceeding on even more specific details, *e.g*., Masson *et al*. (1999) have shown that charged amino acids on one side of α-helix four in domain I of Cry1Aa are involved with passage of ions through the pore. Non-conservative point mutations of Cry1Ab α-helix seven resulted in proteins that were not readily degraded while more conservative alterations affected the ion channel activity (Alcantara *et al*., 2001). Gerber and Shai (2000) showed that the hairpin loop of α-helix four and α-helix five inserted into the membrane and lined the channel and that α-helix five participated in the oligomerisation of Cry1Ac. However, mutations of residues within α-helix five of Cry1Ab showed that this helix was involved in pore formation and the stability of the toxin but not in oligomer formation (Nunez-Valdez *et al*., 2001).

The Cyt δ-endotoxins have a different mechanism of membrane insertion. Instead of forming small pores/channels in the midgut membranes as do the Cry proteins, *in vitro* data suggest that Cyt1A induces permeability *via* a detergent-like perturbation of the membrane (Butko *et al*., 1997; Manceva *et al*., 2005). Structural analysis of Cyt2A showed it is composed of two outer layers of α-helices around a β-sheet structure, and further studies using mutants with reduced toxicity show that the molecular components in the β-sheet are responsible for both membrane binding and pore formation (membrane disruption) (Li *et al*., 1996; Luo *et al*., 1997; Du *et al*., 1999). The β-strands are long enough to span the hydrophobic region of a membrane (Li, 1996). Studies with the similar Cyt1A indicate that the toxin disrupts the membrane through assembly of several monomers within the membrane mediated by two of the α-helices (Gazit *et al*., 1997). An analysis of the structure of Cyt δ-endotoxins suggests that the surface helix hairpins first peel away, exposing the beta-strands, which can then disrupt the membrane (Li, *et al*, 2001). Recent research suggests that a monomer of Cyt2Aa1 binds and inserts into the membrane. Then the monomers that are close to each other bind together into oligomers and form large pores (Promdonkoy and Ellar, 2003).

Tests on binding and pore formation in insect midgut membranes *in vitro* have suggested that receptor binding and pore formation are predictive of *in vivo* toxicity to susceptible insects. However, some very active δ-endotoxins *in vitro* had less activity *in vivo* suggesting that other mechanisms may contribute to the full toxic activity (Peyronnet *et al*., 1997). This may be due to differences in toxin solubility or proteolysis, or experimental limitations. The pH of the insect midgut, which varies among insect species, can affect pore formation as shown with experiments with *Manduca sexta* midgut membranes *in vitro* (Tran *et al*., 2001; Carroll *et al*., 1997). Cry1C-induced permeability was much less at high pH than for Cry1Ac which correlates with *in vivo* studies demonstrating this insect is less susceptible to Cry1C than to Cry1Ac. Another possible mechanism resulting in the observed variation in insect susceptibility to δ-endotoxins is that many insects have mechanisms to repair some damage caused by cell lysis. Moreover, the amount of repair an insect is capable of is dependent on a number of factors such as genetics of the insect host, host age, dosage and potency of the toxins ingested, and various environmental factors (Bauer, 1995). If repair cannot be accomplished, the target insect dies within 2 - 3 days, usually due to bacterial septicaemia (Broderick *et al*, 2006). The insect hemolymph provides a rich nutrient source for various invading bacteria. Microbial Bt insecticides have shown a synergistic effect of combining *B. thuringiensis* spores along with the δ-endotoxin proteins, presumably leading to more rapid bacterial septicemia, although in some cases, spores do not appear to affect toxicity levels (Liu *et al*., 1998).

5. Expression of δ-endotoxin genes in plants

Several papers over the past two decades have examined the introduction and expression of *B. thuringiensis* δ-endotoxin genes in various plants, *e.g*. Mendelsohn *et al*. (2003). The first regulatory

review of a plant pesticide product field release by EPA was in 1986 of a transgenic tobacco plant producing a δ-endotoxin from a *B. thuringiensis* isolate identified at that time as subsp. *berliner*. There are a number of limitations to obtaining high levels of expression of the prokaryotic δ-endotoxin genes in the eukaryotic plant cells basically due to the differences in the transcription and translation systems between eukaryotes and prokaryotes. Differences in transcriptional regulation, mRNA stability, preferential codon usage, and translation efficiency have led to the frequent use of modified *B. thuringiensis cry* genes for insertion in plants (as reviewed by De la Riva and Adang, 1996). Recent work has found that insertion of genes into plant chloroplasts may result in much higher expression levels. One experiment demonstrated that Bt *cry2*Aa2 operon in chloroplasts resulted in toxin being expressed at a level of 45.3% of soluble protein in the leaves. They noted formation of insecticidal crystals (De Cosa *et al*., 2001).

5.1. Methods of gene insertion

A number of recombinant DNA technologies are available for engineering plants for insect resistance through insertion of the *B. thuringiensis* δ-endotoxin genes (De la Riva and Adang, 1996). The most commonly used method has been bacterial mediated transformation using the plant pathogenic bacterium, *Agrobacterium tumefaciens* that causes crown gall disease in plants (Gasser and Fraley, 1989; Cheng *et al*., 2004). Another commonly used plant transformation method consists of direct gene transfer *via* microprojectile bombardment.

5.2. Promoters

Initial attempts at producing *B. thuringiensis* transgenic plants were conducted using full-length protoxins or truncated versions of the *cry* genes under the control of constitutive promoters. Although truncated versions of *cry* genes containing the N-terminal fragment proved more successful than the full-length protoxins, expression of the toxin protein was quite low. Currently, a number of different promoters are available to drive expression of Bt genes in both a spatial and temporal manner (Potenzaa *et al*., 2004). The most commonly used promoter is the cauliflower mosaic virus 35S promoter (CaMV 35S). This constitutive promoter has better activity in dicotyledonous plants than in monocotyledons, although it was used in commercial maize constructs. Other strong or constitutive promoters for monocotyledons include the rice actin 1 promoter, the synthetic maize *Emu* promoter, and the maize polyubiquitin 1 promoter (De la Riva and Adang, 1996).

5.3. Expression levels in plants

Detailed publications on transgenic plants containing the δ-endotoxins from *Bacillus thuringiensis* first appeared in the late 1980's (Barton *et al*., 1987; Fischoff *et al*., 1987; Vaeck *et al*., 1987). Early attempts of engineering the plants containing the full-length protoxin led to very low levels of expression of the δ-endotoxins that was inadequate for proper pest control. Truncated δ-endotoxin consisting only of the active N-terminal portion rather than the full-length protoxin without further modification are still not efficiently expressed, although higher levels of activity against insect pests are seen (Vaeck *et al*., 1987; Koziel *et al*., 1993). Levels of δ-endotoxin up to 0.02% of the total leaf-soluble protein from tobacco and tomato have been reported with the use of truncated toxins (Kumar *et al*., 1996). Shivakumar *et al*. (1986), as reported by De la Riva and Adang (1996), obtained an expression level of only 0.0001% using the full-length protoxin, but a level of 0.07% of the total leaf protein in tobacco leaves with a recombinant truncated version of *cryIAb*. (*cry1Ab*) Generally, maximum levels of the Cry proteins for most U.S. registered plant pesticide products were in the 0.001% (dry weight) range (see Table 2).

Expression levels of the δ-endotoxins in plants, even with the use of truncated toxins, can be quite variable and are dependent on a large number of factors. Expression levels can be enhanced through

modification of the *cry* gene sequences to make them more compatible with plant transcription and translation systems (Perlak *et al*., 1991). In a review article, De la Riva and Adang (1996) suggest the following modifications of the *cry* gene sequences for increasing expression of the δ-endotoxins in transgenic plants: (1) change to preferential codon usage of the plant by eliminating CG and TA dinucleotides at codon positions two and three, and conserving the AT base composition typical within the plant, (2) modify sequences that could lead to mRNA instability or degradation including polyadenylation signals, termination sequences, or splicing sites, (3) reduce regions of mRNA known to form hairpins and other secondary structures of mRNA known to reduce the speed of ribosome translocation, (4) optimise the ATG consensus flanking nucleotides for protein translation (and termination), and (5) introduction of plant viral untranslatable mRNA leader to improve translation initiation.

A major limitation to efficient gene expression in plants results from organisational differences between prokaryotic and eukaryotic genes. For example, coding regions of eukaryotic genes are separated by non-coding regions known as introns, which are generally not present in prokaryotes. In addition, the *B. thuringiensis cry* genes have been shown to be very AT- rich compared to plant coding genes. AT- rich regions in plants are often contained in the non-coding introns, or have a regulatory role in polyadenylation. The AT- rich regions in the *cry* genes may contribute to RNA instability in the plant either through acting as polyadenylation signals or as termination sequences for RNA-polymerase. In addition, poly ATTTA sequences in the *cry* genes can serve as mRNA degradation signals. Other AT sequences can signal incorrect mRNA splicing (De la Riva and Adang, 1996).

Besides the overall greater percentage of A and T bases in the δ-endotoxin prokaryotic genes, there is also a difference in codon usage preference between prokaryotes and eukaryotes that leads to inefficient expression of the δ-endotoxin genes in plants. Whereas the *B. thuringiensis cry* genes often have A or T as the third base of the codon triplets, plants tend to have G or C. Due to the lower number of AU recognizing tRNAs in plants, the speed of translation and synthesis of the δ-endotoxin proteins is reduced if the high AT codon of the bacterial gene is inserted in plants (De la Riva and Adang, 1996).

In addition, there may be tissue and temporal variation in expression. For example, variable expression of Cry1Ac was found between eight cotton hybrids tested in India and the expression also declined consistently as the plant aged, raising concerns for efficacy (Kranthi *et al*., 2005). Other experiments showed a strong decline, occurring as the plant ages, in Cry1Ac expression in cotton (Greenplate, 1999) and Cry1Ab expression in maize (Dutton *et al*., 2004).

5.4. Variable expression in plant parts

Current techniques in genetic engineering may allow specific control of the δ-endotoxins expression in different parts of the plant. Most of the transgenic products developed to date, however, exhibit a wide range of expression in the various plant parts assessed (Table 2). Strong constitutive promoters such as CaMV 35S generally result in production of the gene product under their control (*e.g.* δ-endotoxins) in all the tissues of the plant. However, the CaMV 35S promoter does not seem to express well in pollen as evidenced by low expression seen in maize pollen (Kozeil *et al*., 1993) and relatively low expression in cotton pollen (Greenplate, 1997). Other constitutive promoters such as the CaMV 4AS1 promoter used in MON 863-maize (Cry3b), express well in pollen. Other transgenic plants have restricted expression of the δ-endotoxin to specific plant tissues through the use of tissue-specific promoters. For example, in maize, a promoter derived from the phosphoenolpyruvate carboxylase gene was used to promote expression of *cryIAb* (*cry1Ab*) in green tissue (Hudspeth and Grula, 1989). Tissue-specific transgenic tobacco has been developed by insertion of the δ-endotoxin in the chloroplasts resulting in high levels of expression (McBride *et al*., 1995). In addition, a promoter derived from a maize calcium-dependent protein kinase gene was used to get expression of the δ-endotoxin only in pollen (Estruch *et al*., 1994).

These selective-expression technologies may turn out to be particularly useful to direct exposure toward the target insect and/or to restrict exposure to non-target insects.

Table 2. An example of variation in expression levels of δ -endotoxin in different maize constructs expressing five different δ -endotoxins

Cry Protein Tissue Expression*

Active Ingredient/ OECD Unique ID	Leaf	Root	Pollen	Seed	Whole Plant
Cry1Ab SYN-BT11-1	3.3 ng/mg	2.2-37.0 ng/mg protein	< 90 ng Cry1Ab/ g dry wt. pollen	1.4 ng/mg (kernel)	–
Cry1Ab MON-00810-6	10.34 ng/mg	–	< 90 ng Cry1Ab/ g dry wt. pollen	0.19-0.39 ng/mg (grain)	4.65 ng/mg
Cry1F DAS-01507-1	56.6 - 148.9 ng/mg total protein	–	113.4 - 168.2 ng/mg total protein or 31 to 33 ng / mg pollen	71.2 - 114.8 ng/mg total protein	803.2 - 1572.7 ng/mg total protein
Cry3Bb1 MON-00863-5	30-93 ng/mg	3.2-66 ng/mg	30-93 ng/mg	_	13-54 ng/mg
Cry34Ab1 DAS-59122-7	5 – 302 ng/mg dry weight	24 – 102 ng/mg dry weight	63 – 88 ng/mg dry weight	29 – 85 ng/mg dry weight	9 – 88 ng/mg dry weight
Cry35Ab1 DAS-59122-7	2 – 113 ng/mg dry weight	1 – 16 ng/mg dry weight	0 – 0.2 ng/mg dry weight	1 – 2 ng/mg dry weight	1 – 16 ng/mg dry weight

* Information in table was provided directly by companies at time of data submission in the absence of specific format requirements, resulting in the differences seen among rows. Data are provided only to show variance among tissues within plants (*i.e.*, in rows).

6. Risk assessment of δ-endotoxins in plants

6.1. General issues

The use of *Bacillus thuringiensis* δ-endotoxins in transgenic plants poses some of the same kinds of risk concerns as the use of microbial Bt preparations containing the same δ-endotoxins in terms of understanding the potential toxicity to non-target organisms. The US registered the first micro-organism (*Bacillus popilliae)* for pesticidal use in 1948 and the first *Bacillus thuringiensis* microbial pesticide was registered in 1961. The regulatory system that evolved in the years since then was based on the assessment system for conventional chemical pesticides. The risk assessment framework that has been used for the Bt plants has been influenced by experiences with microbial and chemical pesticides, as well as the extensive experience in evaluating transgenic crops (Mendelsohn *et al*., 2003; Romeis *et al*., 2006a; Romeis *et al*., 2006b). As in chemical risk assessment (described in Commission Regulation

(EC) 1488/94), the assessment of Bt plants must consider both their potential for producing hazardous effects and the exposure to susceptible organisms resulting from the dissemination and persistence of the toxin.

The literature concerning potential hazards that might be caused by the microbial forms of *B. thuringiensis* is not necessarily relevant to predicting hazards from plant-produced δ-endotoxins in plants because the microbial strains can produce other toxins. However, the registered microbial pesticidal strains of *Bacillus thuringiensis* are virtually non-toxic to mammals, and generally show low toxicity to non-target terrestrial and aquatic species (USEPA, 1998). In those instances in which toxicity to non-targets unrelated to the target species, *i.e.*, daphnia species, has been demonstrated, the toxicity has been attributed to other toxins produced by the micro-organism rather than to the δ-endotoxins (USEPA, 1998). Furthermore, the δ-endotoxins from the microbial spore/crystal preparations have historically appeared to be rapidly degraded in the environment. Therefore, the negligible toxicity to non-target organisms and the low persistence allowed for a conclusion of negligible risk for the registered *B. thuringiensis* microbial pesticides (USEPA, 1998). More recent studies on persistence of δ-endotoxins from both plants and micro-organisms indicate that they may bind to some substances in the soil, thus increasing the duration of their presence in the soil (Saxena and Stotzky, 2000; Stotzky, 2000; Crecchio and Stotzky, 2001; Saxena *et al*., 2002a, 2002b), but no adverse effects have been observed in this increase in exposure. Engineering of the δ-endotoxins into plants reduces one aspect of the risk over that of the naturally-occurring microbial forms of *B. thuringiensis* because it eliminates the potentially toxic exotoxins that are produced by some strains of *B. thuringiensis*. At the same time the use of engineered Bt crops may present a longer and higher level of exposure of δ-endotoxins in a truncated active form as compared to the application of conventional Bt preparations. The current information available from studies on long-term cultivation of Bt toxin-expressing plants on residual toxin levels in soil indicate they are not present at detectable levels and do not appear to build up over time (Sanvido *et al*., 2006).

The scientific literature has some examples of tests for effects on non-target organisms including humans, but these tests were conducted with microbial preparations that may have contained multiple toxins. In recent years there have been many publications describing the effects of isolated *B. thuringiensis* δ-endotoxins on both target and non-target insects (see the *Bacillus thuringiensis* toxin specificity database, van Frankenhuyze and Nystrom, 1999). These proteins have also been extensively studied *in planta* (Bhatti *et al*., 2005a, 2005b; Bitzer *et al*., 2006; Daly and Buntin, 2005; Dively, 2005; Head *et al*., 2005; Lopez *et al*., 2005; Naranjo, 2005a, 2005b; Naranjo *et al*., 2005; Pilcher *et al*., 2005; Prasifka *et al*., 2005; Torres and Ruberson, 2005; Whitehouse *et al*., 2005).

There are also numerous studies that are submitted by private companies or testing laboratories to support regulatory decisions for engineered products (Mendelsohn *et al*., 2003; Romeis *et al*., 2006b). While this information is less easily obtained than public literature, it is still useful to discuss in the current context. It should also be stressed that any information provided by companies to facilitate regulatory decisions must meet rigorous data quality standards. These standards are referred to as Good Laboratory Practices (GLP) and are codified in national regulations (US Code of Federal Regulations, 40 CFR 160; EU Directives 87/18/EEC and 88/320/EEC) and described by international organisations such as the OECD (GLP, 2006).

Annex I contains brief summaries of studies related to toxicity received by USEPA in support of Cry1Ab, Cry1Ac, Cry3A, Cry9C, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1, and Cry35Ab1 δ-endotoxins as used in plant products. The tables in the Annex are presented as examples of the kinds of specific data that are reviewed to support regulatory decisions and do not contain the detailed information available from the actual decision documents. Data on other δ-endotoxins are periodically received and their reviews will be available to the public by means of the various national and international databases. Many, if not most of these studies in the tables, have also been submitted to other countries for

evaluation and have been used in international review of these products. The opinions based (in part) on these studies by the EU scientific committees can be found on the internet at http://www.efsa.eu.int/science/gmo/gmo_opinions/catindex_en.html and the Scientific Committee on Plants at http://europa.eu.int/comm/food/fs/sc/scp/outcome_gmo_en.html, and http://europa.eu.int/comm/food/fs/sc/scp/outcome_en.html#opinions. At least one country (the UK) has a web site, http://www.defra.gov.uk/environment/gm/regulation/index.htm, listing scientific regulatory opinions that have used some or all of these studies. In addition, the United States Regulatory Agencies Unified Biotechnology Website lists decisions about reviewed crop plants (http://usbiotechreg.nbii.gov/) and the International Biosafety Clearinghouse (http://bch.biodiv.org/decisions/default.shtml) keeps a database that gives access to decisions on transgenic plants in many countries.

The exposure to the δ-endotoxin relates to the kind of plant containing the δ-endotoxin and the potential for transfer of the δ-endotoxin to other plants. Various δ-endotoxins have been field tested in many crops including maize, potatoes, cotton, soybeans, peanuts, alfalfa, broccoli, cranberries, eggplants, rapeseed, rice, tomatoes, tobacco, and walnut, spruce, apple, and poplar trees (OECD BioTrack Database of Field Trials: http://webdomino1.oecd.org/ehs/biotrack.nsf; Biosafety Clearinghouse: http://bch.biodiv.org/decisions/default.shtml). Commercial uses include δ-endotoxins in potatoes, maize, and cotton (http://www.epa.gov/pesticides/biopesticides).

Risk considerations for Bt crops are based on the same risk assessment process as for other transgenic crops. Potential adverse changes in the plant are considered in the context of increased weed potential and adverse environmental effects. The potential for increased weediness in the transgenic plant should be evaluated as well as the potential for the transgene to increase weediness of wild relatives by cross breeding between the transgenic plant and wild relatives. Outcrossing of δ-endotoxin genes to wild relatives may also produce unintended exposure to susceptible insects that are not pests. In this case, as well as in the case of the cultivation of Bt plants in general the potential exposure to endangered species must also be considered. In addition, a continuous exposure to *B. thuringiensis* δ-endotoxins presents the possibility for sublethal effects on insects and/or development of insects that are no longer susceptible to the lethal effects of the δ-endotoxins.

6.2. Human health assessment

6.2.1. Acute toxicity

Throughout several decades of use of commercial microbial *B. thuringiensis* products, mammalian toxicity has been evaluated. The toxicological database on *B. thuringiensis* shows no mammalian health effects attributable to δ-endotoxins. A review of numerous infectivity and pathogenicity studies indicates a pattern of clearance of the *B. thuringiensis* organisms from rodents after oral, pulmonary, or intravenous challenge (McClintock *et al*., 1995a, 1995b). No significant adverse health effects attributable to the test microbe have been reported in these studies in either body weight gain or mortality by clinical observations, or through examination of the test animal's internal organs at necropsy. To support the Bt plant risk assessments, acute toxicity testing has been performed on rodents using Cry1Ab, Cry1Ac, Cry9C, Cry3A, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1, and Cry35Ab1 (DEKALB, 1997; Monsanto and Novartis, 1996a; Monsanto, 1995a, 1995b; 2001a, 2001b, 2001c; Mycogen and Novartis, 1995c, 1995d; Plant Genetic Systems, 1998c; Mycogen and Pioneer, 2001d, 2001e; 2005b). For all these studies, since maximum levels of toxin were needed for a feeding assay, the toxin was produced in an engineered microbial culture since sufficient amounts of pure toxin could not be extracted from the plants to supply a standard limit dose. This approach required an analysis of the microbially-produced toxin to show that the toxin was sufficiently similar to that produced in the plant using a range of techniques including SDS-PAGE, Western blot, ELISA, N-terminal amino acid sequencing, Glycosylation, and Bioactivity (host range). In all cases, these δ-endotoxins showed no adverse effects at high doses in the range of 3760 to 5220 mg/kg in mice *via* an oral route of exposure. The European

Food Safety Authority (EFSA) evaluated the food safety of δ-endotoxin expressed in maize plants such as Cry1Ab in Bt11 (EFSA, 2005a), Cry1F in maize 1507 (EFSA, 2005b), Cry3Bb1 in MON 863 (EFSA, 2004), and hybrids derived from Cry1Ab and Cry3Bb1 in MON 810xMON 863 (EFSA, 2005c). Short term feeding/toxicity studies on poultry, pigs, calves and cattle also provided additional information on the behaviour of Cry1Ab protein in the gastro intestinal tract (Jennings *et al*., 2003; Chowdhury *et al*., 2003; Einspanier *et al*., 2004; Lutz *et al*., 2005). Cry1Ab was not completely degraded in the gastro-intestinal tract and fragments of the gene and/or immunoreactive protein fragments were still present in the intestinal content and in the faeces, but no residual DNA/protein could be found in animal tissues nor in the peripheral blood, nor was any risk associated with these findings.

The mode of action of *B. thuringiensis* δ-endotoxins in susceptible insects is well-known and was discussed previously in Section IV of this document. Within the insect midgut, the δ-endotoxins bind to unique receptor sites on the cell membrane, causing development of pores, disruption of osmotic balance, and ultimately septicaemia (Gill and Ellar, 2002; Broderick *et al*., 2006). There are no known equivalent receptor sites in mammalian species which could be affected (Noteborn *et al*., 1995). Additional factors that support an insect specific mode of action are the reliance of the lepidopteran midgut on unique ATPases for potassium influx regulation and insect midgut's unique susceptibility to ionic stress (Knowles, 1994), plus the observations that even when Cry toxin binding site proteins are expressed in mammalian cells, the mammalian cells are unable to express the proteins in a form that allows the toxins to bind to the cells (Keeton and Bulla, 1997). The more acidic environment of the mammalian gut also leads to degradation of Cry proteins.

Several recent studies have found some toxicity from several strains of microbial *B. thuringiensis* in immunocompromised mice and severely-stressed mice infected with influenza virus. However, the authors attribute the effects to *Bacillus* toxins other than the δ-endotoxins (Hernandez *et al*., 1998; Hernandez *et al*., 1999; Hernandez *et al*., 2000). This is further supported by the close mapping of the strain that was associated with a human tissue infection to strains of *Bacillus anthracis* indicating that this particular bacillus, in addition to having a plasmid that expressed the characteristic δ-endotoxin crystal, also expressed a mammalian toxin similar to the very potent tripartite *B. anthracis* toxin (Hill *et al*., 2004). Another *B. thuringiensis*-like strain lacking δ-endotoxins showed mammalian toxicity that appeared to be due to haemolytic toxins (Salamitou *et al*., 2000).

6.2.2. Food allergenicity

In the absence of a suitable animal model to predict food allergenicity, a screening model was recommended by a conference entitled "Scientific Issues Related to Potential Allergenicity in Transgenic Food Crops"(FDA Docket 94 N-0053, document TR-1) held April 18-19, 1994, in Annapolis, Maryland. The participants, who were expert food allergy researchers, recommended that new proteins be evaluated by determining their similarity to characteristics of known food allergens. Specifically, the questions to be considered were, does it have a similar amino acid sequence, is it resistant to enzymatic and acid degradation, is it heat stable, is it found in high amounts in edible plant parts, and is it of the appropriate molecular size? Of these criteria, the δ-endotoxins tested to date do not share similar amino acid sequences with known proteinaceous food allergens. Cry1Ac, Cry1Ab, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1, and Cry35Ab1 were shown to be heat labile (Monsanto, 1995b; 2001a, 2001b; 2002c; Monsanto and Novartis, 1996b; Mycogen and Pioneer, 2001f, 2001g; 2005c, 2005d, 2005e, 2005f). The resistance to enzymatic and acid degradation of each of the δ-endotoxins in commercial products has been analysed with a protein digestibility study on the pure gene product (DEKALB, 1997; Herman *et al*., 2003; Monsanto and Novartis, 1996b; Monsanto, 1995a, 1995b; 2001a, 2001b; 2002a, 2002c, 2002d, 2002e; Mycogen and Novartis, 1995c; Mycogen and Pioneer, 2001h; 2005b; Plant Genetic Systems, 1998c). These studies are performed using simulated gastric (acid and pepsin) and intestinal fluids (trypsin at neutral pH) as described in the United States Pharmacopeia (USP, 1995). The degradation process may be tracked by disappearance of a band on SDS-PAGE or assayed using susceptible insects.

The active form of the δ-endotoxin, of course, is resistant to trypsin digestion, but of the δ-endotoxins commercialised to date, all (Cry1Ac, Cry1Ab, Cry1F, Cry3A, Cry2Ab2, Cry3Bb1, Cry34Ab1, and Cry35Ab1), except Cry9C, have been readily inactivated by the digestibility studies. The Cry9C δ-endotoxin passed part of the allergenicity screen because it shows no homology to known toxins or allergens, however it was resistant to degradation by proteases, pepsin at pH 2.0 and to heat at 90°C for 10 minutes (Plant Genetic Systems, 1998c). As a result, Cry9C products were removed from the market, but no instances of human allergenicity have been found although some Cry9C had entered the food supply (see paragraph 67, below). The European Food Safety Authority (EFSA) evaluated the potential allergenicity risk of δ-endotoxin expressed in maize plants such as Cry1Ab in Bt11 (EFSA, 2005a), Cry1F in maize 1507 (EFSA, 2005b), Cry3Bb1 in MON 863 (EFSA, 2004), and hybrids derived from Cry1Ab and Cry3Bb1 in MON 810xMON 863 (EFSA, 2005c). The allergy risk evaluation of Cry proteins has been completed using different approaches, which led to indirect evidence for an allergenicity risk being very low. This included the absence of known allergenicity of the source, absence of significant sequence homology with known allergens and rapid and extensive degradation by pepsin. To date, despite extensive scientific scrutiny no methodology has been found to conclusively assess the potential for dietary allergenicity if a substance does not pass the screening tests.

Bernstein *et al*. (1999) is sometimes cited as support for the potential for δ-endotoxins to be food allergens. However, this research was designed to examine the potential for farm workers to develop reactions and/or antibodies to microbial forms of *B. thuringiensis* products following inhalation exposure, not dietary exposure to δ-endotoxins. Furthermore, the authors did not find a significant reaction to a preparation of δ-endotoxins in the group of workers that exhibited an immune response to whole microbial *B. thuringiensis* extracts (as shown by skin prick testing, no allergy or clinical symptoms were ever seen). The protoxin preparation for this test was derived from the commercial strain of Javelin to which the workers were exposed. The authors concluded that "... it is unlikely that consumers would develop allergic sensitivity after oral exposure to transgenic foods (*e.g.* tomatoes, potatoes) that currently contain the gene encoding this protein." A similar conclusion can be drawn from Siegel (2001).

6.2.3. Human exposure

The primary significant human exposure to δ-endotoxin in plants is by the oral route for food crops. Exposure to the aerosols produced during the processing of material (*e.g.* seed) of Bt plants is an additional, although small, route of human exposure. Many countries require pesticide residue studies to determine the maximum levels of chemical pesticides in or on raw agricultural commodities. Due to the lack of mammalian toxicity for the δ-endotoxins tested at very high doses, these traditional pesticide residue studies are not necessary. Microbial *B. thuringiensis* pesticides that are registered in countries that require tolerances (a.k.a Maximum Residue Levels) have been given an exemption from the requirement for setting a numerical tolerance (MRL). However, an analysis of δ-endotoxin expression levels in various parts of the plant is useful for analysis of non-target organism effects as well as issues related to insect resistance management. These data show that the transgenic plant pesticides in current commercial use have relatively low levels of δ-endotoxins in edible plant parts.

6.2.4. Human risk assessment

The acute oral toxicity data on Cry1Ab, Cry1Ac, Cry9C, Cry3A, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1, and Cry35Ab1 supports the prediction that the Cry proteins would be non-toxic to humans. When proteins are toxic, they are known to act *via* acute mechanisms and at very low dose level (Sjoblad *et al*., 1992). Therefore, since no effects were seen in the acute tests, even at relatively high dose levels, these δ-endotoxin proteins are not considered toxic to humans. Both the long history of safe use of *B. thuringiensis* and the acute oral toxicity data allow for a conclusion that these and other δ-endotoxins pose negligible toxicity risk to humans. The one aspect of human health concern identified in their

assessments was the potential for the Cry9C protein to be a food allergen. Cry9C was conditionally registered in the U.S. for animal feed uses only, with restrictions on cultivation to provide containment. However some unintentional mixing occurred probably either in the field through pollination or after harvest at grain handling facilities and resulted in low levels of the toxin appearing in a few processed maize products. The registration was subsequently withdrawn at the company's request. Studies by the U.S. Food and Drug Administration and the Centers for Disease Control and Prevention did not reveal any cases of human allergenicity attributable to exposure to Cry9C. One individual who showed possible allergenicity to the Cry9C protein by self-administered oral doses and one skin test volunteered for a fully controlled, double-blind, test in a medical centre which proved that he was not allergic to Cry9C protein (Sutton *et al*., 2003). The overall safety record for Bt has been established in laboratory and field studies, which have looked at both formulated Bt sprays and specific Bt genes *in planta* (Betz *et al*., 2000; Siegel, 2001; Federici, 2002).

6.3. Non-target species

6.3.1. Effects on non-target organisms

Effects studies for non-target organisms are designed to determine the actual hazard to a test species, usually using high doses to ensure a margin of safety and certainty and to give a maximum hazard result (Rose, 2006; Romeis *et al*., 2006a). Exposure and assessment studies (including field studies that incorporate both exposure and effects in the same study) will be found in the subsequent non-target species sections of this document. A substantial number of lab-based effects studies have been submitted in support of commercial products. Tests include acute, sub-acute, and reproductive dietary tests for δ-endotoxins in plants on non-target species, preferably those with a history of survival under laboratory conditions. The test substance was the δ-endotoxin expressed in the particular plant tissue expected to be involved in the non-target exposure, or, if it could not be incorporated as such in the diet of the test organism, it was the pure δ-endotoxin. In the US non-target species were generally an avian species (bobwhite quail), a rodent (mouse and/or rat) and a wide range of unrelated non-target insects (honeybees and predacious beneficial insects such as parasitic wasps, ladybird beetles, and green lacewings) selected as representative species that fill some functional or surrogate role and have been demonstrated to survive under laboratory conditions. Laboratory tests on other non-target insects are developed as needed. There have also been numerous six week feeding studies done with broiler chickens. Effects on non-target mammals can partly be assessed by using the acute dietary studies that were performed for human health effects analysis. Aquatic species (fish, *e.g.* rainbow trout, and aquatic invertebrates, *e.g.* daphnia) testing may be useful if they are likely to be exposed, but often, there may be no significant aquatic exposure from substances produced in transgenic plants with the exception of transgenic Bt rice. Studies have also been performed on soil organisms, *e.g.* collembola, which are involved with detritus degradation, and earthworms. The number of soil organisms tested however is limited.

6.3.1.1. Effects on non-target mammals

Data available on laboratory rodents on microbial forms of *B. thuringiensis* do not indicate that there are adverse effects of *B. thuringiensis* preparations on the test animals (*e.g.* USEPA, 1998). Tests with cattle or swine, representing mammals with different digestive systems, are rare and not focused on long-term effects. However, due to the previous mentioned specific mode of action of δ-endotoxins, effects on non-target mammals can be considered quite unlikely. This conclusion is supported by the lack of effects observed for purified δ-endotoxins tested on rodents in support of commercial use of transgenic plants (Annex I). Furthermore, as previously mentioned, there are no known equivalent receptor sites for binding of the δ-endotoxins in mammals (Noteborn *et al*., 1995; Gill and Ellara, 2002; Broderick *et al*. 2006). The mode of action also appears to be insect specific due to the reliance of the lepidopteran midgut on unique ATPases for potassium influx regulation and the insect

midgut's unique susceptibility to ionic stress (Knowles, 1994), plus the observations that even when Cry toxin binding site proteins are expressed in mammalian cells, the mammalian cells are unable to express them in a form that allows the toxins to bind to them (Keeton and Bulla, 1997; Gill and Ellar, 2002; Broderick *et al*., 2006).

6.3.1.2. Effects on avian species

Acute and subchronic testing of northern bobwhite quail and mallard duck has demonstrated that microbial products using *B. thuringiensis* are not toxic or pathogenic (*e.g.* USEPA, 1998). Acute avian oral studies on the actual δ-endotoxins have been submitted in support of commercial use. No effects have been seen from a dietary exposure to bobwhite quail for crops containing Cry1Ab, Cry1Ac, Cry3A, Cry9C, Cry1F, Cry2Ab2, and Cry3Bb1 (DEKALB, 1997; Monsanto and Novartis, 1996c; Monsanto, 1995a, 1995b; 2001c; 2002a; Mycogen and Novartis, 1995c; Plant Genetic Systems, 1998c; Mycogen and Pioneer, 2001e). Additionally, no effects have been observed from a dietary exposure to poultry for maize expressing the binary toxin Cry34Ab1 and Cry35Ab1 (Mycogen and Pioneer, 2005c).

6.3.1.3. Effects on freshwater fish

Microbial products using *B. thuringiensis* have not demonstrated toxicity or pathogenicity to bluegill sunfish or rainbow trout, both freshwater fish. Aqueous LC_{50}'s ranged from 8.7×10^9 to 4.6×10^{10} cfu/L (USEPA, 1998). Cry1Ab was tested in corn meal as 100% of the diet in a catfish assay with no effects seen at the maximum dose tested (>200 ppm) (Monsanto and Novartis, 1996d). Cry2Ab2 and Cry3Bb1 were tested in catfish at dietary levels of 20% w/w cottonseed meal and 35% w/w corn meal respectively with no effects seen (Monsanto, 2001c, 2001d; 2002a). Cry34Ab1 and Cry35Ab1 proteins were tested on rainbow trout for eight consecutive days as a standard fish diet containing a mixture of 100 mg/kg of a mixture of the two Bt proteins with no adverse effects (Mycogen and Pioneer, 2005g).

6.3.1.4. Effects on freshwater invertebrates

Toxicity testing of registered microbial products identified as *B. thuringiensis* subspecies *kurstaki* and *israelensis* demonstrated moderate toxicity to the freshwater invertebrate *Daphnia magna*. Reported LC_{50}'s were in the range of 5 to 50 ppm. A high level of toxicity was shown by *B. thuringiensis* to daphnia with EC_{50}'s in the range of 0.8 to 3 ppm (USEPA, 1998). The toxicity, however, was shown to be unrelated to δ-endotoxins, but rather was a result of heat-labile soluble substances in supernatant fluids. The toxicity is also not attributable to the heat-stable β-exotoxin. The expression of well-characterised δ-endotoxin proteins alone in plants mitigates concerns about toxicity caused by exotoxins or other metabolites produced by various subspecies of *B. thuringiensis* during fermentation for traditional Bt products. Tests using Cry1Ab (Mycogen and Novartis, 1995c), Cry9C (Plant Genetic systems, 1998c), Cry1F (Mycogen and Pioneer, 2001e), and Cry3Bb1 (Monsanto, 2002a) expressed in pollen showed no effects on daphnia. Although corn pollen is assumed to be too large for ingestion by daphnia, tests have reported daphnids becoming yellow in colour internally when exposed to corn pollen (Monsanto, 2002a). Despite the uncertainty of ingestion, these studies are still useful since the major aquatic exposure from most plants expressing δ-endotoxins would be by pollen deposition and these studies serve to rule out these effects on daphnia. In addition, no adverse effect were observed when daphnia were exposed to the Cry34Ab1/Cry35Ab1 proteins at a target concentration of 100 mg protein L^{-1} water (Mycogen and Pioneer, 2005g)

6.3.1.5. Effects on estuarine and marine animals

Toxicity studies of several subspecies of the microbial form of *B. thuringiensis* demonstrated that *B. thuringiensis* subspecies *kurstaki, israelensis,* and *aizawai* are not toxic or pathogenic to grass shrimp, sheepshead minnows, or copepods (*e.g.* USEPA, 1998). Similar tests using δ-endotoxins expressed

in plants have not been required by regulatory agencies because there would be no significant exposure from the plants that have been assessed to date.

6.3.1.6. Effects on earthworms

Studies using the δ-endotoxins found in commercial plant products observed no effects for earthworms dosed with Cry1Ab (Monsanto and Novartis, 1996d; Mycogen and Novartis, 1995a), Cry1Ac (DEKALB, 1997), Cry3A (Monsanto, 1995b), Cry9C (Plant Genetic Systems, 1998c), Cry1F (Mycogen and Pioneer, 2001e, 2001c), Cry2Ab2 (Monsanto, 2001c), Cry3Bb1 (Monsanto, 2002a, 2002f), or Cry34Ab1/Cry35Ab1 (Mycogen and Pioneer, 2005h). Adult *Lumbricus terrestris* fed with Bt corn litter (Cry1Ab, Bt11) showed no significant difference to earthworms fed with non-Bt litter during the first 160 days of the experiment in a single worst-case laboratory study. However, after 200 days Bt-fed earthworms had a significant 18% weight loss compared to a weight gain of 4% of the control. There was no difference in mortality between the Bt and the non-Bt treatment (Zwahlen *et al*., 2003a). The authors concluded that "Further studies are necessary to see whether or not this difference in relative weight was due to the Bt toxin or other factors discussed in the study". Vercesi *et al*. (2006) found no negative impact of Bt maize on important life-history traits of *Aporrectodea caliginosa*, an earthworm species abundant in agricultural soils. Considering all available studies the predominant weight of evidence gives no indication for harmful effects of Bt maize on earthworms. Field studies have been performed and are referenced in the risk section since they incorporate both hazard (effects) and exposure (see section 6.4.1).

6.3.1.7. Effects on non-target insects

Because the δ-endotoxins are used as an insecticide, extensive testing has been performed on pest insects related to those known to be affected by the toxins. In order to be susceptible, non-target insects must have specific receptor sites to which δ-endotoxins can attach and must have the proper midgut pH and enzymatic conditions so that pores are formed in the midgut membranes. Insects are the primary targets of Bt-toxins, and therefore the main non-target organisms that need to be considered for the risk assessment of well-characterised Cry δ-endotoxins and proteins closely related to them. The studies for effects testing in this section (1) will consider only those studies that are designed to report hazard and don't reflect field exposures. Full risk studies, primarily field testing, will be reported in Section 3 Risk Assessment.

As expected from the selective mode of action, registered microbial products incorporating *B. thuringiensis* subspecies *kurstaki, israelensis,* and *tenebrionis* which contain different Cry and Cyt toxins that affect various lepidopteran and/or Diptera insects, were shown to have little to no toxicity to the non-target indicator species for insects, *e.g.* neuropterans, hymenopterans, and coleopterans. These same *B. thuringiensis* subspecies were also minimally toxic to honey bees. However, U.S. registered microbial products identified as *B. thuringiensis* subspecies *aizawai* have been shown to be highly toxic to honey bees (LE_{50} = 15 ppm) (*e.g.* USEPA, 1998). This toxicity was attributed to a heat labile exotoxin, not the δ-endotoxins. A table in appendix 4 of Glare and O'Callaghan (2000) has a list of 92 studies of the effects of various strains of *B. thuringiensis* on 24 Families in nine Orders of beneficial insects (predators of insects, and parasitoids). Only about eight of the effects reported could be judged as harmful to the predator, and that activity might well be attributable to toxins other than δ-endotoxins. The vast majority of studies reported no adverse effects.

Generally, as previously described in this document, the δ-endotoxins have a relatively high specificity. Bt toxins for this reason can be assumed to affect fewer non-target organisms than conventional chemical pesticides, where it is assumed that insecticides will kill most non-target insects. A review by Dutton *et al*. (2003) on risk assessment for entomophagous arthropods acknowledges that the comparison to the effects of a conventional chemical pesticide can be used as an argument for

not requiring testing on non-target herbivores, but suggests that it would be useful to have information on the effects on some non-target herbivores because of the season-long expression of the δ-endotoxins in the plants. Most of the research on the host range of the δ-endotoxins has been conducted on potential pest insects in order to see if those toxins can control them. For example, the Cry2A toxins seem to be highly species-specific, exhibiting insecticidal activity toward lepidopteran and some dipteran species only (van Frankenhuyzen and Nystrom, 1999 database, version 24 January 2002). A considerable number of tests have been performed using Cry2Aa toxins on insect species from the Orders Lepidoptera, Diptera, Coleoptera, Orthoptera, Hymenoptera, Homoptera, Neuroptera, Hemiptera, Isoptera, the insect relatives Collembola and the Crustacean order Isopoda (Crickmore *et al*., 1998).

On the other hand, species more closely related to the target pest species may well be affected by Bt toxins. For genetically engineered crops effects often depend on the specific event. Forest spray uses may affect some species of leptidopterans related to the pest species (Miller, 1990; Johnson *et al*., 1995; Wagner *et al*., 1996) which is why the US Forest Service does not use *B. thuringiensis* spray products where endangered leptidopterans may be present and some lepidopterans have been reported to be affected in and immediately adjacent to Bt maize fields (Zangerl *et al*., 2001). Larvae of the butterfly species, *Danaus plexippus*, *Papilio polyxenes* and *Pseudozizeeria maha* were affected negatively when feeding on pollen of the Bt maize event 176 (Cry1Ab) which may be deposited on their host plants if they are growing in close association with the maize plants (Losey *et al*., 1999; Hansen Jesse and Obrycki, 2000; Hellmich *et al*., 2001; Wraight *et al*., 2000; Zangerl *et al*., 2001; Shirai and Takahashi, 2005). Also, Felke and coworkers showed in laboratory studies that the consumption of pollen of Bt176 maize (Cry1Ab) has adverse effects on the larvae of several European non-target lepidopteran species, although some could be considered pests, *e.g. Plutella xylostella* (diamondback moth), while others such as *Nympahlis io* are protected in certain European regions (Felke and Langenbruch, 2001, 2003; Felke *et al*., 2002). In general, pollen consumption of Bt176 pollen had a negative effect on survival of larvae, their consumption rate, body weight and development time. The LD50 values were 61 – 80 applied pollen grains of Bt176 maize for *Nymphalis io*, 19 pollen grains for *Pieris rapae* and 139 pollen grains for *Pieris brassicae*, but the actual LD50 value is lower as the larvae did not consume all of the applied pollen (Felke and Langenbruch, 2001, 2003; Felke *et al*., 2002).

Many of the above tests were conducted with young larvae (often neonate), however, older larval stages are less susceptible to Bt maize pollen consumption (Felke and Langenbruch, 2001, 2003; Hellmich *et al*., 2001; Felke *et al*., 2002). The toxicity of Bt maize pollen depends on the specific event. Bt176 expresses a higher concentration of Cry1Ab in pollen than other events such as MON 810 and Bt11 (Sears *et al*., 2001). In laboratory assays pollen consumption of MON 810 and Bt11 maize had no acute impact on butterfly larvae of *Danaus plexippus*, *Papilio polyxenes,* or *Antheraea pernyi* (Hansen Jesse and Obrycki, 2000, 2002, Wraight *et al*., 2000, Hellmich *et al*., 2001, Li *et al*., 2005), and impact of these events was considered to be non-existent or negligible. However, in a recent publication Dively *et al*. (2005) demonstrated that a prolonged and natural exposure to MON 810 and Bt11 pollen had adverse effects on larvae of the Monarch butterfly, *D. plexippus*. In laboratory and greenhouse experiments (and field studies, see paragraph 111), larvae exposed to Bt maize pollen had a significantly longer development duration and reduced survival. Also, the resulting pupae and adults showed a lower weight (Dively *et al*., 2004).

In addition to pollen feeding, consumption of maize anthers can also have adverse effects on monarch butterfly larvae causing a lower survival, lower consumption rate, reduced body weight and a longer development time (Hellmich *et al*., 2001; Anderson *et al*., 2004), but later instars were again less affected (Anderson *et al*., 2004). Anderson and coworkers suggested the anther effects may also have been caused by avoidance behaviour of larvae (Anderson *et al*., 2004, 2005) and concluded that there is no risk for the monarch butterfly regarding anther exposure alone due to a low exposure probability in the field (see paragraph 111 in exposure section). However, simultaneous exposure to both Bt anthers and pollen had an additive effect and resulted in a lower survival and consumption rate of monarch

larvae (Anderson *et al*., 2005). Some studies exist on the effects of Bt maize endotoxins other than Cry1Ab on butterfly larvae. Testing the toxicity of Cry1Ac and Cry1F on neonate larvae of the monarch butterfly, Hellmich *et al*. (2001) demonstrated that both Bt toxins were less toxic as compared to Cry1Ab. Mattila *et al*. (2005) tested the effect of pollen consumption from a stacked maize hybrid (Cry1Ab x Cry2Ab2) and of a Cry3Bb1 event on first-instar larvae of the monarch butterfly. Cry3Bb1 had no adverse effects at all, whereas pollen from a Cry1Ab x Cry2Ab2 stack produced both lethal and sublethal effects (Mattila *et al*., 2005). Additional stressors can act in an additive or synergistic way with Bt toxins, thus enhancing the efficacy of δ-endotoxins. For instance, larvae of the European cornborer (Lepidoptera: Crambidae) which had been treated with *B. thuringiensis* formulations and Cry1Ab were less tolerant to protozoan *Nosema* infections than the controls resulting in a higher mortality and stronger negative sublethal effects (*e.g.,* Pierce *et al*., 2001; Reardon *et al*., 2004). For a binary toxin, Cry34Ab1/Cry35Ab1, an insecticidal activity spectrum study which tested the proteins on insects from three orders (Lepidoptera, Homoptera and Coleoptera) and four families (Pyralidae, Chrysomelidae, Aphididae and Noctuidae) demonstrated that only larvae of *Diabrotica* spp. were affected by the Cry34Ab1 and Cry35Ab1 proteins (Mycogen and Pioneer, 2005b).

Toxicity testing of non-target pest insects to determine host range of δ-endotoxins has also been conducted and reviewed (Mendelsohn *et al*., 2003; Rose, 2006; Romeis *et al*., 2006a, 2006b). Dankocsik *et al*. (1990), who reported the isolation of the gene for Cry2Ab, also reported toxicity to Lepidopteran species (*Lymantria dispar*, *Heliothis virescens*, *Trichoplusia ni*, *Helicoverpa zea*, *Ostrinia nubilalis*), but, unlike Cry2Aa, not to a Dipteran species (*Aedes aegypti*), even at a very high dosage. Two studies on a very closely related toxin, Cry2Ab1, showed that it was not active towards Diptera (*Aedes aegypti*) and confirmed its activity against Lepidoptera (*Manduca sexta*) (Widner and Whiteley, 1989, 1990). Laboratory experiments showed that Bt potatoes expressing Cry3Aa had no effect on larval development, longevity and fecundity of the aphid *Myzus persicae* (Kalushkov and Nedved, 2005). The performance of the aphid *Rhopalosiphum padi* feeding on Bt maize (Cry1Ab) was studied in the laboratory by Lumbierres *et al*. (2004). No differences were found on aphid mortality, developmental and pre-reproductive times, fecundity and intrinsic rate of natural increase between the offspring of apterous aphids maintained on Bt or non-Bt maize for several generations. However, the offspring of the first generation of apterous mothers had lower mortality, shorter development and pre-reproductive times, a higher effective fecundity rate and greater intrinsic rate of increase when fed on Bt maize. In contrast the offspring of the first generation of alatae performed better on Bt maize and had a shorter developmental and pre-reproductive time and a higher intrinsic rate of increase on Bt maize (Lumbierres *et al*., 2004). The authors conclude that given these finding, economic effects on maize crops should not be expected (Lumbierres *et al*., 2004).

In contrast with the above reports, the van Frankenhuyzen and Nystrom (1999) database lists a paper by Ahmad *et al*. (1989) reporting that Cry2Ab from *B. thuringiensis* var. *galleriae* is toxic to *Aedes aegypti*, suggesting that the Cry2Ab toxin in Bollgard II could be toxic to Diptera. In contrast, studies by Widner and Whiteley (1989, 1990) showed no toxicity by Cry2Ab2 to Diptera. The apparently contrasting results regarding dipteran toxicity of Cry2Ab could be related to the testing procedures. Dankocsik *et al*. (1990) dissolved the toxin in water and immersed mosquito larvae in the solution while Ahmad *et al*. (1989) immersed larvae in water containing *Bacillus megaterium* expressing the toxin. It is not clear that either of these exposure pathways is appropriate, and there should be a feed-based test of this toxin for dipteran activity if needed to assess the risk to a dipteran species that might be exposed to plant material expressing this particular toxin.

The aphid *Aphis gossypii* showed a shorter reproductive duration and maximum lifespan, lower survival rates and lower potential maximum fecundity on Bt cotton (Cry1A) in the first or second generation (Liu *et al*., 2005a). However, the aphid population soon overcame the negative effects in the second or third generation, and aphids on Bt cotton had longer reproductive durations in the first generation, higher survival rates in the third generation, and longer potential maximum

fecundity. Still, fluctuating asymmetry in three morphological parameters suggests that the stress of cotton on the aphids may have been higher on Bt cotton (Liu *et al*., 2005a). These studies demonstrate that in evaluating Bt effects on non-target herbivores it is important to apply a crop-specific approach, test for lethal and sublethal parameters within, to test several generations and developmental stages of the focus organisms, and to test exposure to the whole Bt plant.

Non-target insect studies have been submitted to support registration of the Bt plants using various δ-endotoxins in assays against several species of representative beneficial insects. These species were chosen in some cases because, as common predators or parasites, they were used for integrated pest management or biocontrol. In other cases, the species have a long history of use in evaluating pesticides. Moreover, they were laboratory-adapted and available for testing using standardised and validated protocols that have been used by many professional laboratories for many years (Rose, 2006; Romeis *et al*., 2006a).

Honey bees (*Apis melifera*) have probably been the most studied non-target insect for the detection of conventional pesticide effects, thus commercial wildlife testing laboratories are very experienced in performing laboratory tests with them, although the new emphasis on detecting effects on honeybee larvae (since δ-endotoxins primarily affect larvae of the target insects) has required some new protocols to be developed. No effects on honeybees have been observed in these new studies submitted in support of the Bt plant registrations. No effects were seen for Cry1Ab and Cry1Ac against honey bees (adult and larvae dosed with toxin and toxin-containing pollen) (Monsanto and Novartis, 1996b; Mycogen and Novartis, 1995c; Monsanto, 1995b). Cry9C in maize pollen showed no effect on adult honeybees or ladybird beetles (Plant Genetic Systems, 1998a, 1998c). Cry3A demonstrated no effects in two honeybee larval studies (Monsanto, 1995a, 1995e). Cry1F showed no effect on honey bees during larval development to adults when exposed to both toxin and toxin-containing pollen (Mycogen and Pioneer, 2001a, 2001c, 2001e,). Similar developmental studies on honey bees from larval stage to adult demonstrated no toxicity from Cry2Ab2 and Cry3Bb1 (Monsanto, 2001c, 2001d; 2002a). The Cry34Ab1 and Cry35Ab1 proteins have no observed adverse effects on honeybee larvae development. No adverse effects were observed on three to five day old larval honeybees when fed with either (i) a single dose of 2 mg of maize pollen expressing the Cry34Ab1 and Cry35Ab1 proteins, (ii) a single dose of 5.6 µg of a mixture of the Cry34Ab1 and the Cry35Ab1 proteins, (iii) a single dose of 3.4 µg of the Cry34Ab1 protein, or (iv) a single dose of 2.8 µg of the Cry35Ab1 protein (Mycogen and Pioneer, 2005i).

The difficulties of developing study protocols using new methodologies can be illustrated by a series of studies that have suggested that Cry1A toxins may have a toxic effect on *Chrysoperla carnea* (lacewing) larvae. Hilbeck *et al*. (1998b) conducted bioassays of purified Cry1Ab toxin on *C. carnea* larvae using two different no-choice feeding strategies. Using direct diet incorporation of 100 µg toxin per ml diet, they observed 57% mortality compared to 30% mortality of the diet only control. Using the other feeding strategy, where toxin free eggs are supplied as the first food source, then larvae are placed onto the diet medium with and without the toxin, the results were 29% and 17% respectively. In another study, Hilbeck *et al*. (1998a) reported that *C. carnea* larvae fed on prey that had been fed on Bt maize (Cry1Ab) had increased mortality rates and slightly increased developmental times. Prey species were target lepidopteran *Ostrinia nubilalis* (European Corn Borer) and non-target lepidopteran *Spodoptera littoralis* (Egyptian Cotton Leafworm) larvae. Averaged across these two prey species (the difference between prey species was not significant), 'Bt-prey' fed *C. carnea* larvae exhibited 62% mortality whereas 'non-Bt-prey' fed *C. carnea* larvae exhibited 37% mortality. In their next study, Hilbeck *et al*. (1999) extend their analysis of prey-mediated effects of the Cry1Ab toxin on the lacewing *C. carnea* by including multiple concentrations of Bt in the prey's food and by comparing the effects of the Cry1Ab toxin, protoxin and the Cry2A protoxin using their bioassay system. They report that *C. carnea* fed on *S. littoralis* reared on the highest concentration of Cry1Ab, 100µg/g of diet, had a mortality rate of 78% compared to the control mortality rate of 26%.

Review of the studies by Hilbeck *et al*. (1998a; 1998b) by the US National Research Council (NRC, 2000) concluded that the effects reported may be due to differences in feeding strategy and amount of toxin supplied. They recommended that field studies be done to assess the effects of Bt crops on natural predators and cited an example of such a study: a two year, relatively small scale field test, that found no differences in natural enemies on Bt and non-Bt corn (maize) crops (Pilcher *et al*., 1997). There has been subsequent field testing at larger scale (see part 3 of this subsection on risk to non-target organisms). In the Hilbeck *et al*. work (1998b; 1999) high levels of toxin were used in no-choice feeding situations for both the lacewing itself (Hilbeck *et al*., 1998b) and the prey species (Hilbeck *et al*., 1999). However, from the studies it is not possible to differentiate between effects mediated *via* the ingestion of the toxin itself or effects mediated *via* a decreased host quality. A translation of the Hilbeck studies into the field is also difficult because behavioural mechanisms such as prey avoidance and alternative prey will need to be considered. A more recent study did not observe direct toxicity of high doses of Cry1Ab on the green lacewing larvae (Romeis *et al*., 2004). Romeis *et al*. (2004) point out that effects on *C. carnea* due to Cry1Ab may be rather due to diet quality effects than due to direct toxic effects. However, difference in experimental design makes direct comparisons of the results between the two studies open to interpretation.

Despite the differences seen in the above studies, their results are valuable in showing the need for research in developing laboratory testing protocols using more representative exposure techniques that better reflect field exposures and involve representative non-target insect species that often are difficult to rear under laboratory conditions. For example, a review by Dutton *et al*. (2003) on risk assessment for entomophagous arthropods recommends an assessment for these predators combining laboratory testing and exposure assessment based on knowledge of their feeding habits, plus field studies, if necessary. Andow and Hilbeck (2004) proposed an integrated ecological whole plant assessment strategy. A USEPA Scientific Advisory Panel (August 7, 2002) concluded that the green lacewing (*Chrysoperla carnea*) dietary testing was complicated by the difficulty of getting an adequate exposure and of laboratory testing with this species. Therefore, the USEPA is now following the recommendation from its advisory panel (SAP) and asks for dietary testing on the minute pirate bug (*Orius insidiosus*) as a more appropriate test species than the green lacewing. *Orius* spp. typically occur in US maize fields as egg predators and typically feed on pollen.

Data for Cry1Ab, Cry1Ac, and Cry3A showed no effect on adult ladybird beetles, green lacewing larvae (direct exposure), and parasitic wasps (Monsanto, 1995a, 1995b; Monsanto and Novartis, 1996a). Cry9C in maize pollen showed no effect on ladybird beetles (Plant Genetic Systems, 1998c). Cry1F fed in toxin form to green lacewing larvae, parasitic wasps, and adult ladybird beetles showed no effects (Mycogen and Pioneer, 2001c, 2001e). When Cry1F was fed to Monarch larvae, no mortality was seen, although there was some growth inhibition seen at the high dose, 30,000 ng/ml diet (Hellmich *et al*., 2001). Cry2Ab2 and Cry3Bb1 toxin studies showed no effect on adult ladybird beetles and green lacewing larvae (Monsanto, 2001c; 2002a). In addition, no effects were seen in a developmental Cry3Bb1 pollen feed study on ladybird beetles from larvae to adults (Monsanto, 2002d), and two Cry3Bb1 pollen feeding studies on two different species of ladybird beetles (Monsanto, 2002b; Duan *et al*., 2002). Similarly Cry34Ab1 and Cry35Ab1 proteins did not show toxic effects on green lacewing larvae (Mycogen and Pioneer, 2005g), parasitic wasps (Mycogen and Pioneer, 2005g) or adult ladybird beetles (Mycogen and Pioneer, 2005b). No effects were seen when ladybird larvae were fed a mixture of 50% corn earworm eggs and 50% maize pollen expressing the Cry34Ab1 and Cry35Ab1 proteins (Mycogen and Pioneer, 2005a).

Short term laboratory studies showed that four lepidopteran species were sensitive to Cry1Ac, but six species of non-target insects and four species of beneficial insects showed no toxic effects after being fed purified Cry1Ac at concentrations 100 times higher than found in the field in pollen and nectar of transgenic cotton (Sims, 1995). In the laboratory, the majority of beneficial natural enemies tested so far showed no adverse effects due to consumption of Cry1A toxin or of transgenic Cry1A plant material,

e.g., Orius spp., *Geocoris* spp., *Cyrtorhinus* spp., *Nabis* spp. and *Zelus* spp. (Heteroptera), and *Coleomegilla* spp. and *Propylea* sp. (Coccinellidae) (*e.g.,* Pilcher *et al*., 1997; Zwahlen *et al*., 2000; Al-Deeb *et al*., 2001; Bernal *et al*., 2002a; Bai *et al*., 2005). Likewise, no adverse effects were detected for Cry3A and Cry3B toxins for *Orius sp.* and *Lygus* spp. (Heteroptera) and *Coleomegilla* sp. (Coccinellidae) (Riddick and Barbosa, 1998; Armer *et al*., 2000; Duan *et al*., 2002; Lundgren and Wiedenmann, 2002; Kalushkov and Nedved, 2005).

Romeis *et al*. (2006b) reported no effects on natural enemies fed directly with Bt plant material, but confirmed that tritrophic effects do occur, *i.e.,* predators and parasitoids may be adversely affected when feeding on Bt-fed prey. Romeis *et al*. (2004; 2006b) attribute the tritrophic effect to inferior nutritional quality of the prey. Ponsard *et al*. (2002) examined the effect of Bt cotton and of lepidopteran prey that had ingested Bt cotton on the survivorship of four important heteropteran predators of cotton pests. Longevity significantly decreased by 27-28% for *Orius tristicolor* and *Geocoris punctipes*, whereas no effect was found for *Nabis* sp. and *Zelus renardii* (Ponsard *et al*., 2002). Consumption of pollen of transgenic Bt rice caused a lower survival in *Propylea japonica* (Coccinellidae) (Bai *et al*., 2005).

Hymenopteran parasitoids often show adverse effects when parasitizing host reared on Bt plants or diets, which is mostly attributed to a reduced quality of the host (cf. Lövei and Arpaia, 2005; Romeis *et al*., 2006b). A laboratory study on soybean loopers (*Pseudoplusia includens*) parasitised with hymenopteran parasites and raised on Cry1Ac cotton showed retarded development that was attributed to possible sublethal effects on the host (Baur and Boethel, 2003). Likewise, *Microplitis mediator*, an important endoparasitoid of the cotton bollworm in China, suffered from reduced survival and growth inhibition when parsitizing *Helicoverpa armigera* raised on Bt cotton leaf powder (Cry1Ac) (Liu *et al*., 2005). *Cotesia marginiventris* (Hymenoptera: Braconidae) survival, development times and cocoon weights were significantly negatively affected if their *Spodoptera littoralis* host larva (Lepidoptera: Noctuidae) had been fed Cry1Ab Bt maize (Vojtech *et al*., 2005). Prütz and coworkers studied the effect of hosts, *Chilo partellus* (Lepidoptera. Crambidae), raised on Bt corn leaf material (Cry1Ab) on the parasitoid *Cotesia flavipes* (Hymenoptera: Braconidae), and parasitoids on Bt-fed hosts suffered under reduced weight and a lower probability to complete their development (Prütz and Dettner, 2004; Prütz *et al*., 2004). The adverse effects on the parasitoid *C. flavipes* had a secondary effect on the fourth trophic level. Female hyperparasitoids *Tetrastichus howardi* (Hymenoptera: Eulophidae) parasitizing *C. flavipes* developing in Bt-fed *C. partellus* had lower body weight and offspring (Prütz *et al*., 2004). Survival and adult size of the parasitoid *Aphidius nigripes* (Hymenoptera: Braconidae) was reduced when developing on non-target aphids fed Bt potato (Cry3A) (Ashouri *et al*., 2001). Bt maize (Cry9C) fed hosts led to adverse effects regarding development time, longevity and mortality of the parasitoid *Parallorhogas pyralophagus* (Hymenoptera: Braconidae), but did not affect sex ratio, egg load, or adult size (Bernal *et al*., 2002b). In conclusion, sublethal impacts on target and non-target herbivores can affect parasitoids and may translate into impacts on the degree of biological control provided by parasitoids by altering parasitoid-host population dynamics, and secondary effects can also include secondary pests or pests in subsequent or neighbouring crops (Bernal *et al*., 2002b). However, sublethal effects also need to be assessed in the context of the role of beneficial insects in the pest-controlled crop and the population dynamics of the respective insects (Mendelsohn *et al*., 2003; Romeis *et al*., 2006b).

Potential effects to ladybird beetles and aphids have been examined with Bt (Cry3Aa) potatoes. In a study by Dogan *et al*. (1996), aphids fed on potato leaves expressing a gene for *Bacillus thuringiensis* var. *tenebrionsis* δ-endotoxin (Cry3 toxins) were force-fed to lady beetle larvae and adults (*Hippodamia convergens*). Since lady beetles are in the insect Order Coleoptera and are thus potential targets of Cry3 toxins, this study was aimed at determining whether these beneficial predatory insects would be affected by feeding on Bt transgenic potato-fed aphids. Results showed no aphid prey-mediated effect on lady beetles. The exact mechanisms for the lack of effect observed in this study are not clear, however, it is known that aphids hardly ingest Bt toxins when sucking on Bt plants (*e.g.,* Head *et al*., 2001; Raps *et al*., 2001; Dutton *et al*., 2002); consequently, prey-mediated effects by aphids are unlikely.

Riddick and Barbosa (1998) detected no adverse effects mediated by the prey *Leptinotarsa decemlineata* feeding on Bt potato (Cry3A) onto the predatory coccinellid *Coleomegilla maculata*.

Two soil arthropods, a collembolan, *Folsomia candida* Willem, and an orbatid mite, *Oppia nitens* Koch, were tested with Cry1Ab and Cry1Ac in cotton and with Cry3A in potato. No adverse effects were seen (L.Yu *et al*., 1997). Collembola studies have been submitted in support of the registrations for all the commercial constructs, Cry1Ab, Cry1Ac, Cry1F, Cry3A, Cry9C, Cry2Ab2, Cry3Bb1, Cry34Ab1, and Cry35Ab1 (see references). No effects were seen for separate studies using plant-produced and microbial-produced Cry1Ac and Cry9C δ-endotoxins (DEKALB, 1997; Novartis and Monsanto, 1996; Plant Genetic Systems, 1998c). There were two apparently contradictory studies that were submitted for registrations of Cry1Ab producing maize products. One study using pure 200 ppm Cry1Ab toxin derived from recombinant *Escherichia coli* had no observable effects on two collembola species (*Folsomia candida* and *Xenylla grisea*) (Novartis and Monsanto, 1996). The other study using lyophilised leaf extract reported mortality to *Folsomia candida* at a level of 125 mg Cry1Ab - maize leaf protein/kg of soil (Mycogen and Novartis, 1995d). It has not been established if the toxicity observed in this study is due to the Cry1Ab δ-endotoxin or to some other protein interactions of the leaf extract. A worst-case assessment can be performed using these hazard data as discussed in paragraph 112. A study using 200 ppm Cry3A microbial-produced toxin showed no effect on *Folsomia candida* and *Xenylla grisea* (Novartis and Monsanto, 1996). No effects were seen in a chronic 28 day study of Cry1F, and dietary studies of Cry2Ab2 and Cry3Bb1 (Mycogen and Pioneer, 2001e; Monsanto, 2001c, 2002a). The woodlouse, *Porcellio scaber* (Crustacea: Isopoda), performed better when fed with Cry1Ab maize as compared to the non-transgenic isoline, which was attributed to a better nutritional quality of the Bt corn (Escher *et al*., 2000). A laboratory study of 16 species of Carabidae ground beetles fed Cry3Bb1 and Cry1Ab in maize pollen found no effects from the Bt toxins (Mullin *et al*., 2005). A maximum hazard dosing laboratory study with an artificial diet containing 930 µg/g of diet of Cry3Bb1 protein showed no adverse effects on the survival, development and growth of the ground beetle, *Poecilus chalcites* (Duan *et al*., 2006). Larvae of *Poecilus cupreus* (Carabidae) fed with prey raised on Bt maize showed a higher mortality than larvae fed with non-Bt prey. These effects may be prey-mediated, however, direct effects cannot be excluded as the carabid larvae did ingest Bt toxin (Meissle *et al*., 2005). A study designed to test the effects of Cry3Bb1 and Cry1Ab maize toward 16 species of Carabidae ground beetles found no effects from the Bt toxins (exposure to pollen) whereas nearly complete mortality was found for seeds treated with neonicotinoid insecticides (Mullin *et al*., 2005).

6.3.2. Exposure to non-target organisms

If adverse effects are seen for an organism in the acute hazard testing, exposure analysis will enable a risk assessment to be performed. Several routes of exposure exist which can be either linked to the exposure from the toxin produced in the crop or the exposure from toxin produced in wild relatives if outcrossing can take place. However, the potential for outcrossing is crop and region specific and is best addressed in the consensus documents for the respective crops. Exposure to non-target organisms depends on the habitat and feeding ecology of the organism and its life stages. Exposure can be either direct *via* the uptake of Bt plant material and δ-endotoxin bound to soil or indirect *via* the food chain. A worst case direct exposure can be estimated from the maximum levels of δ-endotoxin that may be present in the different plant parts. The data submitted in support of the U.S. EPA registration applications showed great variation in toxin concentration for different constructs, tissues, and different ages of the plant. As an example of variation among constructs, Cry1Ab δ-endotoxin protein expression levels were reported for several commercial constructs in maize. One construct showed maximum levels of 10.34 µg/g leaves, 4.65 µg/g whole plant, and <0.09 µg/g pollen (dry weight) (Monsanto, 1995c; 1995d). Another Cry1Ab maize construct showed maximum levels of 4.4 µg/g leaves, 0.6 µg/g whole plants, and 7.1 µg/g pollen (Mycogen and Novartis, 1995d).

Cry3Bb1 expression in another construct showed maximum levels of 450 µg/g leaves, 390 µg/g roots, and 42 µg/g pollen (dry weight).

Some of the highest expression levels were seen for a Cry9C construct in maize (Plant Genetic Systems, 1998c). The highest amounts (on a dry weight basis) seen in the various plant parts (for the vegetative growth stage) were 250.0 µg/g whole plant, 175.0 µg/g tassel, 44.0 µg/g leaves, 25.87 µg/g root, 18.6 µg/g kernel, 2.8 µg/g stalk, and 0.24 µg/g pollen. The amounts of δ-endotoxin declined rapidly as the plant aged and no new protein was produced to replace the protein being degraded. The whole plant δ-endotoxin analysis on a dry weight basis showed 250 µg/g for the vegetative growth stage, 230 µg/g at pollen shed, 96 µg/g at silage, and 22 µg/g at harvest. These exposure numbers could be used directly for organisms that feed on the plants. However, with the exception of pollen feeding insects, those organisms can be considered target pest organisms and are not intended to be protected from the toxin. There are some organisms that feed on other insects as well as plants, *e.g.* heteropteran predators, which could be considered to be both beneficial and potential plant pests. In addition, soil detritivores feeding on decaying transgenic Bt plant material and predators consuming herbivores and detritivores feeding on Bt plants can be also exposed to δ-endotoxins.

Pollen is a potential source for exposure to non-target insects. As described in the effects section, pollen consumption from deposition on plants can affect non-target susceptible insects as well as pest insects (Felke and Langenbruch, 2001, 2003; Felke *et al*., 2002). The majority of the maize fields in Europe shed pollen during July (Zscheischler *et al*., 1990; Lang *et al*., 2004). Usually pollen anthesis continues for 5-8 days, however, under favourable conditions the vast majority of pollen shedding may occur within a 2-day period (Treu and Emberlin, 2000; Wolt *et al*., 2003), but maize fields can shed pollen also up to 10-14 days after the onset of anthesis (Treu and Emberlin, 2000, Oberhauser *et al*., 2001). Considerable amounts of pollen can be shed by maize, and Emberlin *et al*. (1999) estimated maize pollen output to be approximately 70 kg per acre (= 0.4 ha) within a maize field. Maize pollen may be dispersed by wind as far as 800 m (Treu and Emberlin, 2000) or even several kilometre (Brunet *et al*., 2003), but due to their large size and weight only less than 1% of maize pollen grains are deposited further than 60 m away from the "source" field (Raynor *et al*., 1972). In general, the majority of the maize pollen is deposited within 10 meters of the maize field edge as there is an exponential decline of pollen numbers with growing distance from the maize field (Hansen *et al*., 2000; Wraight *et al*., 2000; Stanley-Horn *et al*., 2001; Zangerl *et al*., 2001; Lang *et al*., 2004; Li *et al*., 2005; Shirai and Takahashi, 2005). On average, one third of the maize pollen, which drifted into field margins, was found on the surfaces of butterfly host plants (Pleasants *et al*., 2001; Lang *et al*., 2004). Pollen on butterfly host plants within the range recorded can cause adverse effects on some butterfly larvae if the pollen contains Bt protein(s) active against lepidopteran species and the densities exceed a toxic threshold (Felke and Langenbruch, 2003; Zangerl *et al*., 2001; Dively *et al*., 2004). Knowledge of naturally occurring maize pollen densities on food plants is indispensable for assessing the expected effects of Bt maize on butterfly larvae along field edges, together with the concentration of toxin in the Bt maize pollen, and its toxic effect on butterfly larvae (Lang *et al., 2*004).

Predators consuming prey (or insects consuming honeydew secreted by some insects) feeding on Bt plants are potentially exposed to Bt toxins if the prey (or honeydew) contains the δ-endotoxin. Different prey organisms will differ in the amount of toxin they incorporate. For instance, aphids seem to not (or barely) ingest Cry proteins when sucking on Bt plants such as Cry1Ab corn, presumably because maize phloem sap contains no Bt (Head *et al*., 2001; Raps *et al*., 2001; Dutton *et al*., 2002). In contrast, lepidopteran larvae feeding on Bt maize incorporate Cry1Ab proteins in varying concentrations depending on the species (Head *et al*., 2001; Raps *et al*., 2001; Dutton *et al*., 2002; Vojtech *et al*., 2005). Also, other herbivores feeding on Bt plants contained δ-endotoxins (Cry1Ab, Cry1Ac), *e.g.* a mite, a thrip, a hymenopteran species and a slug (Dutton *et al*., 2002; Howald *et al*., 2003; Obrist *et al*., 2005; Harwood and Obrycki, 2006). In the detritivore *Porcellio scaber* (Isopoda), feeding on decaying Bt maize Cry1Ab toxins could be detected (Wandeler *et al*., 2002). In a laboratory study, a ground beetle

feeding on Bt-contaminated prey incorporated the Bt toxins and exhibited a higher mortality than controls (Meissle *et al*., 2005). Field data demonstrate that non-target herbivores occurring in Bt maize field can take up Bt endotoxins. Harwood *et al*. (2005) showed that Araneae, Coccinellidae, and Nabidae contained on average between 0.42 to 2.53 µg Bt toxin/g fresh weight, while Zwahlen and Andow (2005) were able to measure Bt toxin levels between 6.4 to 117.3 µg/g fresh weight in some Carabidae 6.4 to 117.3 µg/g.

As Bt plants express endotoxins during the whole season, potential tritrophic exposure of predators *via* prey feeding on Bt plants may be increased in comparison to Bt sprays if the Bt sprays are not applied throughout the growing season. The implications of exposure on the performance of these non-target organisms are still not clear; however, the above data show that long-term exposure to Bt toxins can occur in the field. Behavioural characteristics of predators, in particular prey choice, can affect their exposure to Bt endotoxins. In prey choice experiments adult *P. cupreus* (Carabidae) did not avoid Bt containing prey, and even selected Bt-fed *Spodoptera littoralis* (Meissle *et al*., 2005). Another ground beetle, *Lebia grandis*, consumed prey fed with Bt potato leaves (Cry3A) as much as prey fed with non-Bt potato (Riddick and Barbosa, 2000). Larvae of *Chrysoperla carnea* showed a preference for prey fed non-transgenic corn as compared to prey fed Bt corn (Cry1Ab), which would potentially reduce the exposure of this predator (Meier and Hilbeck, 2001). Rovenska *et al*. (2005) showed in laboratory experiments that eggplant expressing the Cry3Bb toxin are preferred by the herbivorous spider mites, *Tetranychus urticae* (Acari). At the same time the predator, *Phytoseiulus persimilis* (Acari), consumed significantly less Bt-fed spider mites.

Bt plant residues remain in the field after harvest. Cry1Ab was still detectable in Bt maize leaves or in the soil of Bt maize fields after the growing seasons, though mostly in low concentrations (Hopkins and Gregorich, 2005; Zwahlen *et al*., 2003b; Baumgarte and Tebbe, 2005). The worst-case exposure to soil organisms can be estimated from the whole plant δ-endotoxin expression levels at harvest. For the Cry9C construct that expressed high levels of toxin (Plant Genetic Systems, 1998c), the amount of δ-endotoxin at harvest is 99 g/acre (assuming that an acre contains 25,000 maize plants) and the expected environmental concentration (EEC) is 0.11 mg/kg in 15cm deep soil**.** A laboratory bioassay submitted in support of a commercial product showed, using a susceptible insect (*Heliothis virescens*), that plant-produced Cry9C δ-endotoxins in test soils biodegraded over 42 days and had a half-life of 4.5 days (Plant Genetic Systems, 1998b). These results are consistent with the half-life of 2 to 46 days reported for Cry1Ac in cotton in a microcosm study (Palm *et al*., 1996). Similarly, for the second Cry1Ab construct described above, if senescent post-harvest maize plants were tilled into the top six inches of soil, there would be a maximum of 4.2×10^{-4} mg Cry1Ab/kg soil (190 mg Cry1Ab/acre x 1/0.5 extraction efficiency x 1 acre (6" deep)/9.08×10^{5} kg soil = 4.185×10^{-4} mg Cry1Ab/kg soil). However, soil δ-endotoxins from *B. thuringiensis* can bind to humic acids, clays, and the organomineral complex found in soil which may give some protection from degradation (see below paragraph 102). Moreover, the distribution of the Bt toxin in the soil may be unevenly distributed as a result of decaying plant material (Baumgarte and Tebbe, 2005; Hopkins and Gregorich, 2005).

Vettori *et al*. (2003) studied the persistence and activity of Bt in soil following application of a commercial Bt spray (FORAY 48B®) against the gypsy moth in oak forests in Sardinia, Italy. The results indicated that *Bacillus thuringiensis kurstaki* and its toxin introduced into soils in sprays can persist for long periods (at least 88 months for *Btk* and at least 28 months for its toxin). One laboratory study of six non-transgenic maize lines and two Cry1Ab lines showed that due to feeding avoidance by a decomposer not affected by the Bt toxin, one of the Bt lines was not degraded as fast as any of the other lines, although there was considerable difference among the non-transgenic lines too (Wandeler *et al*., 2002). One publication (Zwahlen *et al*., 2003a) reported slower degradation for Cry1Ab protein in corn litter in the field as compared to the laboratory and another publication (Zwahlen *et al*., 2003b) reported detection of Cry1Ab in maize leaves buried in bags in the soil and in plant material on the surface for up to 200 to 240 days suggesting that the Cry protein persists in the plants as long as

the plants have not yet been degraded. Recently, the decomposition of different plant species expressing Bt toxins was analysed in laboratory experiments and results were discussed in relation to lignin content and potential environmental consequences. Generally, Bt plants showed lower decomposition rates than non-Bt plants. However, this effect was not clearly related to lignification or reduced microbial activity in soil (Flores *et al*., 2005).

Recent research has suggested that Cry1Ab toxin from Bt corn (Bt maize) is released in root exudates in soil and liquid growth situations (Saxena *et al*., 1999; Saxena and Stotzky, 2000). In the first study, Saxena *et al*. (1999) show that the Cry1Ab in a transgenic corn (Bt maize) crop, truncated to an active form of toxin, is released into the liquid growth medium after seven days and that after 25 days was absent probably due to microbial and/or plant mediated degradation. In this and their subsequent study (Saxena and Stotzky, 2000), they also show that the toxin is released from roots of transgenic maize grown in different soil types. In both cases, Stotzky and co-workers used ELISA and tobacco hornworm larval bioassays to detect the toxin, and in the first study they also used SDS PAGE (protein gel electrophoresis). They suggest that, because these maize plants are expressing a truncated form of the Cry1Ab toxin, thus eliminating the solubilisation and proteolytic processing aspects of toxin specificity, and because there are few field data on the levels of toxin in soils, there may be unintended non-target effects on soil organisms. Soil δ-endotoxins from *B. thuringiensis* microbial cells, as well as those produced from plants, can bind to humic acids, clays, and an organomineral complex found in soil thereby giving some protection from degradation by soil micro-organisms (Saxena and Stotzky, 2000; Stotzky, 2000; Crecchio and Stotzky, 2001; Saxena *et al*., 2002a, 2002b). The toxins can be detected in soil for several months (Tapp and Stotzky, 1995a, 1997), and maintain bioactivity in the laboratory when bound to soil particles (Tapp and Stotzky, 1995b). However, additional laboratory bioassays of plant-produced δ-endotoxin incorporated into natural soil showed a decrease in activity equivalent to the decrease in non-bound toxin that is bio-degraded by soil microbial flora (Palm *et al*., 1996; Pratt *et al*., 1993). For example, the rapid degradation of Cry1F protein in soil has been confirmed using insect bioassays (*Heliothis virescens*) as the analytical quantification method, resulting in a half-life of 0.6 days (Herman *et al*., 2001; 2002b). In a similar way, soil degradation of Cry34Ab1 and Cry35Ab1 was analysed with insect bioassays using southern corn rootworm (*Diabrotica undecimpunctata howardi*), resulting in a half-life of less than four days for this binary toxin (Herman *et al*., 2002a).

If the toxin is actively "exuded" by roots, *i.e.* secreted *via* the cell secretory apparatus, it would likely be present in greater concentrations in the soil than if it were released from 'leaky' cells or from normal plant dynamics such as the shedding ('sloughing') of root tip cells or degradation of some root during overall root growth. This is an important consideration in analysing any risk that arises from these types of transgenic crops (USEPA, 2000). Since the Cry1Ab toxin does not have a signal peptide, a short N-terminal sequence required for secretion in eukaryotic cells, it is not expected to be secreted by plant cells (Vitale and Denecke, 1999). It seems more likely that the source of the ‘exudate’ is shedding ('sloughing') of root tip cells or degradation of some root during overall root growth. This effect may be unique to maize since a multiple year study did not find any Cry1Ac protein in the soil from Bt cotton (Head *et al*., 2002). This phenomenon and the above mentioned experiments do not appear to predict the amount of Bt protein remaining in the soil during active cultivation as evidenced by the fact that multiple field studies did not find any Cry1Ac (Head *et al*., 2002) or Cry1Ab protein in the soil from Bt cotton or corn fields (Dubelman *et al*., 2005). In addition, soils collected during monitoring studies in fields planted with MON810 or Bt11 corn for three or more consecutive years, in five corn-growing areas of the USA, were analyzed using a statistically validated insect bioassay. The Cry1Ab protein was found in soil at only one site, at pollination time, and at levels very near the detection limit (LOD = 0.03 µg/g). This transient residue dissipated soon after harvest. There was no Cry1Ab protein detected in any of the other four sites, or at any other time during or after the corn growing season (Dubelman *et al*., 2005). Soils collected from multi-year field studies of Cry3Bb1 protein in MON863 (YieldGard Rootworm) field plots in Kansas were analyzed using Cry3Bb1 ELISA kits. Only one sample showed

a trace residue of Cry3Bb1 protein (<0.007 μg/g), which dissipated rapidly. There was no soil persistence of the Cry3Bb1 protein and no detectable effects on surface or sub-surface soil arthropods (Ahmad *et al*., 2005). Multi-year monitoring studies of the Cry1Ac protein in several Bollgard fields using insect bioassay (LOD = 0.008 μg/g) and ELISA analysis (LOD = 0.004 μg/g) also showed no detection of the Cry1Ac protein in soil specimens collected three months after tillage (Head *et al*., 2002).

6.3.3. Risk to non-target organisms

Because of the selectivity of the Bt δ-endotoxins, non-target organisms belonging to a similar taxonomic group as the target organisms are those most likely to be affected. Predatory insects can be exposed to the δ-endotoxin in plant parts if their prey feed on the transgenic plant. Their prey, however, may be susceptible to the δ-endotoxin and, in consequence, be of inferior quality or not be available as a diet for the predatory insect. Generally, control of herbivorous crop pests by any sort of pesticide will negatively affect predatory insects by removing their food, even if the pesticide does not directly affect them. Information about predatory insect species and the effect of Bt plants on their populations would also be useful for the purpose of planning integrated pest management if releases of biocontrol insects are to be conducted simultaneously with the use of Bt crops.

Field surveys can be good indicators of overall effects against non-target insects, but are generally difficult to design and control and are expensive to conduct and analyze. The ability to detect changes in the abundance of species or taxa depends much on the experimental design and the statistical power (Lang, 2004). EPA considers field testing for effects on non-target arthropods as a higher tier evaluation that could be required depending on the conclusions from laboratory testing. A Scientific Advisory Panel (USEPA, 2003) concluded that "appropriately chosen single species Tier I laboratory tests showing no detrimental effects are sufficient to make a short term hazard assessment and that field studies be conducted when these tests show toxicity (as higher Tier testing described in the OPPTS Microbial Testing Guidelines) but that proper multi-year commercial field studies with appropriate statistical power are needed to determine long term ecological effects." This allows, for example, for testing on appropriate field plots which avoids the potential sampling errors caused by arthropod movement to and from small plots (Prasifka *et al*., 2005).

Many field tests have now been conducted and most have been published. A field survey of beneficial arthropods (including lady beetles, predacious Carabids, brown lacewings, green lacewings, minute pirate bugs, assassin bugs, damsel bugs, parasitic wasps, damselflies, dragonflies, and spiders) revealed no significant differences in insect numbers between two transgenic Cry1F maize lines and their equivalent non-engineered maize lines, except for some slight variations that had no consistent pattern (Mycogen and Pioneer, 2001e). A two year field study on Cry3Bb1 maize collected a total of 156,572 organisms from 16 orders and 36 families. The invertebrates included pests, predators, parasitoids, detritivores and decomposers. The Bt maize showed no detectable overall effect on the abundance of non-target invertebrates (Monsanto, 2002e; 2002g). As part of a Spanish specific monitoring program for Bt maize (Bt176), a farm-scale study was initiated in the year 2000 to assess the potential impacts of Bt maize on predatory arthropods. The data indicate that Bt maize had no adverse effect on naturally occurring predators (De La Poza *et al.,* 2005) or on certain maize pests including aphids, leafhoppers, cutworms and wireworms (Pons *et al.,* 2005).

Reductions of population densities of specialist predators and parasitoids of *Ostrinia nubilalis* are to be expected as this is the target pest to be controlled in Bt maize fields (Bourget *et al*., 2002). Siegfried *et al*. (2001) found that populations of specific natural enemies of *Ostrinia nubilalis* are less abundant in Bt maize fields than in non-Bt maize fields. In a field test in France, Bt maize had a negligible impact on non-target herbivores or beneficial arthropods collected on the plants throughout the growing season (Candolfi *et al*., 2004). However, results of field studies comparing the effects of Bt maize with insecticide treatments against the target pest show that broad-spectrum insecticides, like pyrethroids,

reduce abundance of a range of predator and parasitoid species not specific to *Ostrinia nubilalis* (Dively and Rose, 2003; Candolfi *et al*., 2004). A three year field test with Cry1Ab and Vip3 maize showed that effects observed in the Bt maize plots were significantly lower than the community disturbances caused by insecticide applications and these changes did not carryover to the following growing season (Dively, 2005). A two year field test of Cry1Ab maize showed a slight decrease in a generalist predator species, *Nabis sp.* (Heteroptera)., but no other non-target phytophagous or predaceous arthropod populations were decreased in the Bt maize plots. It appeared that the nabids, which are not very common in maize plots, were reacting to the reduced numbers of prey (Daly and Buntin, 2005).

No effect was seen on four generalist predators (two coleopterans, one heteropteran, and one neuropteran) of the European Corn Borer in three years of large scale field tests of Cry1Ab maize (lepidopteran-protected) at three sites in Iowa, but *Macrocentrus cingulum*, a European Corn Borer specialist hymenopteran parasitoid was seen at significantly reduced densities in Bt maize as compared to the non-Bt maize. This specialist was shown to be attracted to, and have increases in their population densities in, the non-Bt maize plots (Pilcher *et al*., 2005). A three year field study in Illinois of Cry3Bb1 maize (rootworm-protected) surveyed foliage-dwelling arthropods and found no consistent adverse impact on the relative abundance of any non-target foliage-dwelling arthropod taxon, including predators and parasitoids (140,000 were captured and identified) (Bhatti *et al*., 2005b). A companion three year study on Cry3Bb1 maize in Illinois found no consistent adverse impacts on the abundance of any non-target, ground-dwelling taxon compared with the non-*Bt* isoline. The taxa included Araneae (spiders), Carabidae (ground beetles), Staphylinidae (rove beetles), and detritivores (decomposers), such as Japygidae (diplurans), Lathridiidae (scavenger beetles), Formicidae (ants), Chilopoda (centipedes), and Oligochaeta (earthworms) (Bhatti *et al*., 2005a).

Several field tests completed in Bt cotton fields found no significant effect of Bt cotton on secondary heteropteran pests, aphids and natural enemies (Wang and Xia, 1997; Fitt and Wilson, 2002; Liu *et al*., 2002b; Wu and Guo, 2003; Torres and Ruberson, 2005; Head *et al*., 2005). A six year large scale field study in Arizona on Cry1Ac cotton showed 19% reduction in five of 22 taxa of foliar-dwelling arthropod natural enemies compared with non-*Bt* cotton (Naranjo, 2005a). However a companion five year field study examined whether the Bt cotton had an effect on the natural enemy community's impact on three key pests and found that the potential predator impact was unaltered by Bt cotton but was depressed with insecticide applications, thus indicating that the effects observed in the six year study had little ecological impact (Naranjo, 2005b). In a field study conducted by Sisterson *et al*. (2004), arthropod abundance did not differ between Bt cotton and non-Bt cotton plots, but abundance was lower in pure Bt cotton plots as compared to a row mixture of Bt and non-Bt plants. In a three year field study in Australia, species richness of beneficial arthropod communities were reduced in pesticide sprayed cotton compared to Cry1Ac cotton and non-sprayed cotton. Slightly higher numbers of dipterans, damsel bugs, and jassids were found in conventional, non-sprayed cotton compared to Bt cotton (Whitehouse *et al*., 2005). In a three year field study in China, ladybird beetle numbers were lower in Cry1Ac Bt cotton fields (attributed to reduced number of prey), whereas spider densities increased on Bt cotton. Acarids were not affected by Bt cotton, and the impact on aphids was observed to be inconsistent over years (Men *et al*., 2004). The overall arthropod diversity and the diversity of pest sub-communities were increased, but diversity of natural enemy sub-communities were decreased in Bt cotton (Men *et al*., 2003). Although insecticides were not applied against the main pest (Cotton Bollworm) on transgenic cotton, the total number of insecticide applications in three years was no less than on non-Bt cotton, because additional applications were necessary against piercing/sucking pests on Bt cotton (Men *et al*., 2004). This is in contrast to the situation in Australia where pesticide reduction of 75-85% has been achieved over a ten year period (APVMA, 2003) and key pollution indicators have shown substantial declines in streams and rivers draining cotton growing areas (NSW Dept. Land & Water Conservation, 2001). In another field study in China, the densities of two secondary pest species (Hemiptera: Miridae) did not differ between Bt and non-Bt cotton, however, pest damage by mirids was significantly higher in

unsprayed Bt cotton as compared to non-Bt sprayed cotton, indicating that these mirids have become key pests in transgenic cotton that may require additional control measures (Wu *et al*., 2002). Chinese publications reported that a possible tritrophic adverse effect on natural enemies in the laboratory depended on the Bt cotton variety (Guo *et al*., 2004), that natural enemies increased and phytophagous pests decreased in Bt cotton as compared to non-Bt IPM cotton fields (Liu *et al*., 2002a), and that arthropod predators had generally higher population densities in transgenic Bt cotton field than in non-Bt cotton fields either with IPM or chemical control (Wan *et al*., 2002). A review of field tests published to date concluded that the large-scale studies in commercial Bt cotton have not revealed any unexpected non-target effects other than subtle shifts in the arthropod community caused by the effective control of the target pests (Romeis *et al*., 2006b).

As described above, in the majority of field studies there were no observed effects of Bt plants on invertebrate natural enemies. However, there are some exceptions, some with reduced and some with increased abundance of focus organisms in Bt treatments, of which the following are examples. Jumping spiders (Salticidae) were less abundant in Bt cotton (Cry1Ac, Cry2Aa) (Whitehouse *et al*., 2005), but spiders as a whole were recorded to be more numerous in Bt maize (Cry3Bb), Bt cotton (Cry1Ac, Cry2Aa) and Bt potato (Cry3A) (Riddick *et al*., 2000; Men *et al*., 2004; Bhatti *et al*., 2005b; Men *et al*., 2004). Effects were often found with regards to predacious bugs (Heteroptera) with reduced numbers in Bt fields for Bt cotton (Cry1Ac, Cry2Aa), Bt corn (Cry1Ab) (Daley and Buntin, 2005; Naranjo, 2005a; Whitehouse *et al*., 2005), increased numbers in Bt fields for Bt maize (Cry1Ab) (Musser and Shelton, 2003), and inconsistent varying results in other studies (Wold *et al*., 2001; Reed *et al*., 2001; De la Poza *et al*., 2005). Lacewings (Neuroptera) were found to be less abundant in Cry1Ab × Vip3A cotton (Dively, 2005), and showed an inconsistent pattern in Cry1Ab maize (De la Poza *et al*., 2005). The majority of field studies on Coccinellidae showed no or inconsistent Bt crop effects, the exceptions being higher numbers in Bt fields for Cry1Ab maize and Cry3Aa potato (Musser and Shelton, 2003; Pilcher *et al*., 2005) and lower numbers in Cry1Ac cotton and Cry3Bb1 maize (Men *et al*., 2004; Bhatti *et al*., 2005b). Abundance of some parasitoid Hymenoptera was lower in Cry1Ab maize (Dively, 2005; Pilcher *et al*., 2005). However, Bt treatments were sometimes only compared to insecticide treated conventional crops (and not untreated controls), therefore the specific effect of the Bt construct was not studied but only compared to the application of chemical insecticides (*e.g.* Riddick *et al*., 2000; Head *et al*., 2005; Torres and Ruberson, 2005). The density shifts of natural enemies recorded above were often ascribed to prey dynamics or plant-mediated indirect causes. In the context of field tests it is important to be aware that the abundance of insects may be highly variable and influenced by multiple factors. As a consequence, experimental design and sample size are critical to obtain the necessary statistical power so that the probability to detect potential effects is reasonably high (Marvier, 2002).

The pollen of the Bt maize event 176 (Cry1Ab) was shown to cause negative effects in the field on two butterfly species, the Monarch and the black swallowtail (Stanley-Horn *et al*., 2001; Zangerl *et al*., 2001). Bt176 is no longer cultivated in the United States, but is registered in the European community, *e.g.,* with a cultivation area of 32,000 hectares in Spain in 2003 (Lumbierres *et al*., 2004). In the United States an extensive series of research studies to analyse any potential harm to Monarch butterflies was begun following a research letter to *Nature* suggesting that they could be susceptible to pollen from Bt maize (Losey *et al*., 1999). A series of workshops initiated many studies (*e.g.* Hellmich *et al*., 2001; Oberhauser *et al*., 2001; Pleasants *et al*., 2001; Sears *et al*., 2001; Stanley-Horn *et al*., 2001; Zangerl *et al*., 2001). Of these, the studies conducted in the field under normal cultivation practices found no adverse effect of pollen (and of maize anthers) of the events MON 810 and Bt11 on larvae of the Monarch butterfly, *Danaus plexippus*, and the black swallowtail, *Papilio polyxenes* (Wraight *et al*., 2000; Stanley-Horn *et al*., 2001; Jesse and Obrycki, 2003; Anderson *et al*., 2004). In contrast to these results, Dively *et al*. (2004) could demonstrate that Monarch larvae and adults are negatively affected in survival, development, weight and size after continuous and natural exposure to MON 810 and Bt11 during anthesis in the field. Considering both insect sensitivity and exposure, it was concluded that cultivation

of Bt maize expressing Cry1Ab poses no great risk to the Monarch butterfly, because only a minor part of the whole population would be exposed to pollen shedding maize fields in the United States (Mendelson *et al*., 2001; Dively *et al*., 2004). In other areas of the world, exposure of non-target leptidopterans may merit closer scrutiny where agricultural land and natural habitats are more closely integrated.

Only a few field studies on the effect of Bt plants on soil arthropods exist. A field study of the effects of microbial *B. thuringiensis* subsp. *kurstaki* (Dipel ES) on forest soil fauna showed no effect on earthworms, enchytraeids, oribatids, gamasids, and collembolans (Beck *et al*., 2004). Dively (2005) showed no adverse effects of transgenic corn (Cry1Ab × Vip3A) on saprovorous soil arthropods, including springtails and mites, in a three year study. Likewise, Bt maize expressing Cry3Bb1 against corn rootworm had no effects on Collembola, mites and nematodes and other soil-dwelling invertebrates (Al-Deeb *et al*., 2003; Jasinski *et al*., 2003; Ahmad *et al*., 2005; Bhatti *et al*., 2005a; Bitzer *et al*., 2005). Only in the study of Bhatti *et al*. (2005a) were a few effects observed on 2 - 3 taxa out of 14 taxa tested: Chilopoda numbers were slightly lower in Bt corn plots during two years of the three year study, Staphylinidae abundance was lower in Bt plots in one year, and the Bt effect on Diplura varied among years. In a field test in France involving three field of Bt corn (event 176), no statistically significant treatment effects were observed for diversity indices and for behaviour of soil dwelling arthropod taxa throughout the season. (Candolfi *et al*., 2004).

Because of the concern that δ-endotoxins from both the naturally occurring *B. thuringiensis* microbial residency in the soil and from the Bt plants, might persist in the soil (see paragraphs 100, 102 and 103, above), experiments have been performed to assess the effects on soil non-target organisms, including both soil micro-organisms and macro-organisms. The first of these (Saxena and Stotzky, 2001) reported that earthworms, nematodes, protozoa, fungi, and bacteria, including actinomyces were not affected by 40 days in soil planted with Cry1Ab maize or 45 days in soil with added Cry1Ab maize biomass. The toxin was found in the earthworm guts, but was cleared in 2 to 3 days after moving them to non-Bt soil. The earthworm results agree with results of a seven year field trial with a strain of *B. thuringiensis* subsp. *kurstaki* where the microbial Bt was shown to germinate in three species of earthworm and one tipulid larvae with no harm noted to the organisms (Hendriksen and Hansen, 2002). The earthworms and other soil organisms seem to provide a soil niche for replication of the many subspecies of *B. thuringiensis* that can account for the widespread distribution of *B. thuringiensis* in soils worldwide. No mortality was observed in earthworms fed Cry1Ab maize litter in a 200 day study in the laboratory and the field, although there was some unexplained weight loss after 200 days for the adults, see also paragraph 74 73 (Zwahlen *et al*., 2003a). A laboratory study with Dipel 176 in microbial microcosms concluded that it would be unlikely that Bt would have a significant impact on the non-target microflora under field conditions (Visser *et al*., 1994). In addition to the 2001 Saxena and Stotzky publication, there have been a number of more recent publications that found no significant effects of Bt plants on soil microflora (Dunfield and Germida, 2004; Motavalli *et al*., 2004; Blackwood and Buyer, 2004 (effects seen "are small"); and Devare *et al*., 2004).

It should be noted that it is difficult, if not impossible, to adequately assess any risk associated with any changes in soil microflora. The soil microflora is extremely variable according to type of soil, temperature, moisture, plant growth, nutrients, pH, and many other factors which may vary between locations abut also within a single plot and over very small distances. The soil food web structure varies with climate and geography (Neher, 1999). Cultivation and planting monocultures of agricultural crops has a major impact on the composition of the soil microflora. Furthermore, the microbial populations are very resilient. Even after intentional chemical fumigation, as with methyl bromide, the soil micro-organisms regrow rapidly. Measuring microbial mediated reactions is a more general way to assess soil population activity, but the effects of changes in these are also not fully understood. A recent review (Nannipieri *et al*., 2003) of the state of knowledge of soil microbial diversity notes that generally a reduction in any group of microbes results in other micro-organisms taking over the previous

group's function because of the redundancy inherent in microbial activities. It also cautions against using the newer community analysis techniques without critically considering their limits. The question of whether some change is an adverse effect or a beneficial effect is likely to depend on the context of the question and may often not have an answer, which also cautions against generalisations of results.

6.4. Other ecological issues

6.4.1. Loss of effectiveness of biological control of weedy species.

Wild relatives of crop plants that have weedy characteristics may become protected from insects released as classical biological control agents if they acquire and express a δ-endotoxin gene from the related crop. It is unlikely that a biological control insect would be intentionally used for this purpose since it would probably also be a pest of the crop plant. However, the naturally-occurring crop pest insects might also be contributing to reducing the impact of related weeds. The potential for increasing weediness has been studied in sunflower and rape plants. Wild varieties of sunflower (*Helianthus annuus*) can be a weed in agricultural settings. Cultivated sunflower is known to hybridize frequently with wild sunflower in the western and midwestern United States. Snow *et al*. (2003) studied a *cry1Ac* gene in backcrossed wild sunflower populations. Lepidopteran damage on transgenic plants was strongly reduced relative to control plants at their two study sites, while damage by several weevil and fly species was unaffected. The results suggest that reduced herbivory (by lepidopteran species but not other herbivores) caused transgenic plants to produce an average of 55% more seeds per plant relative to non-transgenic controls at the field site in Nebraska. A similar but non-significant trend was seen at the site in Colorado (14% more seeds per plant). In a greenhouse experiment the transgene had no effect on fecundity, suggesting that it was not associated with a fitness cost. If Bt sunflowers are released commercially, the authors expect that Bt genes will spread to wild and weedy populations, limit damage from susceptible herbivores on these plants, and increase seed production when these herbivores are common. In other experiments, Bt oilseed rape has been shown capable of hybridising with wild relatives in the lab and in the field. Greenhouse experiments have suggested there may be a fitness advantage conveyed by the Cry1Ac but field studies have not yet been done to confirm this (Halfhill *et al*., 2002; Vacher *et al*., 2004).

The potential for outcrossing is a critical part of an assessment of this kind of risk. As previously mentioned outcrossing potential is very crop and region specific and is best addressed in the consensus documents for the crop. As a mitigation measure, various engineering or planting strategies could be used to reduce or eliminate the potential for out-crossing to wild relatives if they occur in proximity to areas in which the transgenic crops are grown.

6.4.2. Potential for adverse effects on endangered or threatened species

The risk to non-target species, especially endangered species, should be considered in a risk assessment. Any endangered species site restrictions on the use of conventional chemical insecticides would be an indication that the potential for adverse effects from the more specific δ-endotoxins should be evaluated. Testing has shown that δ-endotoxins are relatively specific, *i.e.,* they do not affect all the species within any given order. In the case of plants expressing Cry1 or Cry3 proteins effects on endangered Lepidoptera or Coleoptera therefore are the major concern and the risk assessment should consider if there is likely to be an exposure to rare or endangered species. Although potential effects will focus on agricultural habitats a transfer of the Bt toxin *via* pollen to adjacent habitats needs to be considered. This is especially the case in structured landscapes such as parts of Europe where agricultural land is in close proximity to, or part of, nature conservation sites or ecologically sensitive areas (Lang, 2004). In the United States larvae of 229 lepidopteran species feed on host plants associated with maize (Losey *et al*., 2003). According to Schmitz *et al*. (2003) seven percent of the German Macrolepidopteran species mainly occur in arable land and are potentially exposed by Bt maize pollen. The study showed

that over 39% of these 97 species are rare or endangered. The authors advised implementing a risk related monitoring plan for species of concern in the EU. Wolt *et al*. (2005) suggest a stepwise approach to monitoring where a thorough risk assessment is conducted based on the trait, the crop plant in which it is expressed including the spatial and temporal pattern of expression, factoring in the receiving environment to determine the need for monitoring or mitigation procedures.

Any potential for outcrossing also needs to be considered in the assessment of risks to rare or endangered species. The introgression of the Bt trait to wild relatives would considerably increase the exposure and may lead to the spread of the Bt trait into non-managed habitats (Snow *et al*., 2003). Letourneau *et al*. (2003) listed 502 species of Lepidoptera worldwide that feed on cotton, rapeseed and rice or their wild relatives, and which would be exposed and potentially at risk if Bt plants would escape or outbreed.

6.4.3. Potential for loss of efficacy.

Up to now, Bt resistant lepidopteran pest species like *Ostrinia nubilalis* or *Sesamia nonagrioides* have not been found in fields in Europe (Evans, 2002; Bourguet *et al*., 2003; Farinós *et al*., 2004). Although laboratory tests showed that maize borer populations are capable of developing some degree of tolerance to the Cry1Ab protein (Huang *et al*., 2002), laboratory selection and F2 screening to generate highly resistant *O. nubilalis* strains have not been published so far (Bourguet, 2004). However, another lepidopteran pest (*Plutella xylostella*) has developed resistance to Bt toxins in the US (Tabashnik *et al*., 2003). Large scale cultivation of Bt crops over several years could increase the selection pressure on pest species, which might result in the development of resistance (Fox, 2003). This could have several consequences including the use of alternative phytosanitary measures to control the pest including the use of insecticides other than Bt toxins. The likelihood of occurrence is low since, under field conditions and several years of cultivation, no resistance has been reported. However, it is difficult to predict future responses of pest populations. Therefore, if long term efficacy is a concern, potential target pest resistance development could be monitored during Bt crop cultivation. In addition, or as an alternative, methods have been developed that may be used to prevent or delay the development of insect resistance in the field (Williams *et al*., 1992; Rajamohan *et al*., 1998; Matten, 1998; Pittendrigh *et al*., 2004).

References

Ahmad, A., C. Nicholls, and D.J. Ellar. 1989. Cloning and expression of an entomocidal protein gene from *Bacillus thuringiensis galleriae* toxic to both lepidoptera and diptera. FEMS Microbiol. Lett. 59:197-202.

Ahmad, A., G.E.Wilde, and K.Y. Zhu. 2005. Detectability of coleoptera-specific Cry3Bb1 protein in soil and its effect on non-target surface and below-ground arthropods. Environ. Entomol. 34:385-394.

Al-Deeb, M.A., G.E. Wilde, and R.A. Higgins. 2001. No effect of *Bacillus thuringiensis* corn and *Bacillus thuringiensis* on the predator *Orius insiduosus* (Hemiptera: Anthocoridae). Environ. Entomol. 30:625-629.

Al-Deeb, M.A., G.E. Wilde, J.M. Blair, and T.C. Todd. 2003. Effect of Bt corn for corn rootworm control on non-target soil microarthropods and nematodes. Environ. Entomol. 32:859-865.

Alcantara, E.P., O. Alzate, M.K. Lee, A. Curtiss, and D.H. Dean. 2001. Role of α-helix seven of *Bacillus thuringiensis* Cry1Ab δ-endotoxin in membrane insertion, structural stability, and ion channel activity. Biochemistry 40:2540-2547.

Alcantara, E.P., R.M. Aguda, A. Curtiss, D.H. Dean, and M.B. Cohen. 2004. *Bacillus thuringiensis* delta-endotoxin binding to brush border membrane vesicles of rice stem borers. Arch. Insect Biochem. Physiol. 55:169-177.

Andow, D.A. and A. Hilbeck. 2004. Science-based risk assessment for non-target effects of transgenic crops. BioScience 54:637–649.

Anderson, P.L., R.L. Hellmich, M.K. Sears, D.V. Sumerford, and L.C. Lewis. 2004. Effects of Cry1Ab-expressing corn anthers on monarch butterfly larvae. Environ. Entomol. 33:1109–1115.

Anderson, P.L., R.L.Hellmich, J.R. Prasifka, and L.C. Lewis. 2005. Effects on fitness and behaviour of monarch butterfly larvae exposed to a combination of Cry1Ab expressing corn anthers and pollen. Environ. Entomol. 34:944-952.

AOGTR (Australia Office of the Gene Technology Regulator). 2002. The biology and ecology of cotton (*Gossypium hirsutum*) in Australia. 30 p. Available from: http://www.ogtr.gov.au/pdf/ir/biologycotton.pdf

APVMA (Australian Pesticides and Veterinary Medicines Authority). 2003. Evaluation of the new active *Bacillus thuringiensis* var. *kurstaki* delta-endotoxins as produced by the *cry1Ac* and *cry2Ab* genes and their controlling sequences in the new product Bollgard II Cotton Event 15985. 44 p. Available from: http://www.apvma.gov.au/publications/prsbollgard2.pdf

Armer, C.A., R.E. Berry, and M. Kogan. 2000. Longevity of phytophagous heteropteran predators feeding on transgenic Btt-potato plants. Entomol. Exp. Appl. 95:329-333.

Aronson, A. 1995. The protoxin composition of *Bacillus thuringiensis* insecticidal inclusions affects solubility and toxicity. Appl. Environ. Microbiol. 61:1057-4060.

Aronson, A.I. and Y. Shai. 2001. Why *Bacillus thuringiensis* insecticidal toxins are so effective: unique features of their mode of action. FEMS Microbiol. Lett. 195:1-8

Ashouri, A., D. Michaud, and C. Cloutier. 2001. Recombinant and classically selected factors of potato plant resistance to the Colorado potato beetle, *Leptinotarsa decemlineata*, variously affecting the potato aphid parasitoid *Aphidius nigripes*. BioControl 46:401-418.

Bai, Y.Y., M.X. Jiang, and J.A. Cheng. 2005. Effects of transgenic cry1Ab rice pollen on fitness of *Propylea japonica* (Thunberg). J. Pest Sci. 78:123-128.

Ballester, V., F. Granero, R.A. de Maagd, D. Bosch, J.L. Mensua, and J. Ferre. 1999. Role of *Bacillus thuringiensis* toxin domains in Toxicity and receptor binding in the diamondback moth. Appl. Environ. Microbiol. 65: 1900-1903.

Banks, D.J., J.L. Jurat-Fuentes, D.H. Dean, and M.J. Adang. 2001. *Bacillus thuringiensis* Cry1Ac and Cry1Fa δ-endotoxins binding to a novel 110 kDa aminopeptidase in *Heliothis virescens* is not N-acetylgalactosamine mediated. Insect Biochem. Mol. Biol. 31:909-918.

Barjac, H. de, M. Sebald, J. Charles, W.H. Cheong, and H.L. Lee. 1990. *Clostridium bifermentans* serovar *malaysia* une nouvelle bacterie anaerobie pathogene des larves de moustiques et de simulies. C.R. Acad. Sci., Paris 310:383-387.

Barloy, F., M.M. Lecadet, and A. Delecluse. 1998. Distribution of clostridial cry-like genes abong *Bacillus thuringiensis* and Clostridium strains. Curr. Microbiol. 36:232-237.

Barton, K.A., H.R. Whiteley, and N.S. Yang. 1987. *Bacillus thuringiensis* δ-endotoxin expressed in transgenic *Nicotiana tabacum* provides resistance to lepidopteran insects. Plant Physiol. 85:1103-1109.

Bates, S., J.-Z. Zhao, R. Roush, and A. Shelton. 2005. Insect resistance management in GM crops: past, present and future. Nat. Biotechnol. 23:57-62.

Battisti, L., B.D. Green, and C.B. Thorne. 1985. Mating system for transfer of plasmids among *Bacillus anthracis, Bacillus cereus,* and *Bacillus thuringiensis*. J. Bacteriol. 162:543-550.

Bauer, L. S. 1995. Resistance: A threat to the insecticidal crystal proteins of *Bacillus thuringiensis*. Fla. Entomol. 78:414-443.

Baumgarte, S. and C.C. Tebbe. 2005. Field studies on the environmental fate of the Cry1AB Bt toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. Mol. Ecol. 14:2539-2551.

Baur, M.E. and D.J. Boethel. 2003. Effect of Bt-cotton expressing Cry1A(c) on the survival and fecundity of two hymenopteran parasitoids (Braconidae, Encyrtidae) in the laboratory. Biol. Control 26:325-332.

Beck, L., J. Rrombke, A. Ruf, A. Prinzing, and S. Woas. 2004. Effects of diflubenzuron and *Bacillus thuringiensis* var. *kurstaki* toxin on soil invertebrates of a mixed deciduous forest in the Upper Rhine Valley, Germany. Eur. J. Soil Biol. 40:55-62.

Beegle, C.C. and T. Yamamoto. 1992. Invitation Paper (C.P. Alexander Fund): History of *Bacillus thuringiensis berliner* research and development. Can. Entomol. 124:587-616.

Berliner, E. 1911. Über die Schlaffsucht der Mehlmottenraupe. Z. gesamte Getreidewesen 3:63-70.

Berliner, E. 1915. Über die Schlaffsucht der Mehlmottenraupe (Ephestia kühniella Zell.) und ihren Erreger *Bacillus thuringiensis*, n.sp. Z. angewandte Entomologie 2:29-56.

Bernal, C.C., R.M. Aguda, and M.B. Cohen. 2002a. Effects of rice lines transformed with Bacillus thruingiensis toxin genes on the brown plant hopper and its predator *Cyrtorhinus lividipennis*. Entomol. Exp. Appl. 102: 21-28.

Bernal, J.S., J.G. Griset, and P.O. Gillogly. 2002b. Impacts of developing on maize-intoxicated hosts on fitness parameters of a stem borer parasitoid. J. Entomol. Sci. 37:27-40.

Bernhard, K., P. Jarrett, M. Meadows, J, Butt, D.J. Ellis, G.M. Roberts, S. Pauli, P. Rodgers, and H.D. Burges. 1997. Natural isolates of *Bacillus thuringiensis*: Worldwide distribution, characterization, and activity against insect pests. J. Invertebr. Pathol. 70:59-68.

Bernstein, I.L., J.A. Bernstein, M. Miller, S. Tierzieva, D.I. Bernstein, Z. Lummus, M.K. Selgrade, D.L. Doerflier, and V.L. Seligy. 1999. Immune responses in farm workers after exposure to *Bacillus thuringiensis* pesticides. Environ. Health Perspect. 107:575-582.

Betz, F.S., B.G. Hammond, and R. Fuchs. 2000. Safety and advantages of *Bacillus thuringiensis* protected plants to control insect pests. Regul. Toxicol. Pharmacol. 32:156-173.

Bhatti, M.A., J. Duan, G. Head, C.Jiang, M.J. McKee, T.E. Nickson, C.L. Pilcher, and C.D. Pilcher. 2005a. Field Evaluation of the Impact of Corn Rootworm (Coleoptera: Chrysomelidae)-Protected Bt Corn on Ground-Dwelling Invertebrates. Environ. Entomol. 34:1325-1335.

Bhatti, M.A., J. Duan, G. Head, C.Jiang, M.J. McKee, T.E. Nickson, C.L. Pilcher, and C.D. Pilcher. 2005b. Field Evaluation of the Impact of Corn Rootworm (Coleoptera: Chrysomelidae)-Protected Bt Corn on Foliage-Dwelling Arthropods. Environ. Entomol. 34:1336-1345.

Bitzer, R.J., M.E. Rice, C.D. Pilcher, C.L. Pilcher, and W.F. Lam. 2005. Biodiversity and Community Structure of Epedaphic and Euedaphic Springtails (Collembola) in Transgenic Rootworm Bt Corn. Environ. Entomol. 34:1346-1376.

Blackwood, C.B. and J.S. Buyer. 2004. Soil microbial communities associated with Bt and non-Bt corn in three soils. J. Environ. Qual. 33:832-836.

Bourguet, D. 2004. Resistance to *Bacillus thuringiensis* toxins in the European corn borer: what chance for Bt maize? Physiol. Entomol. 29:251-256.

Bourguet, D., J. Chaufaux, A. Micoud, M. Delos, B. Naibo, F. Bombardes, G. Marque, N. Eychenne, and C. Pagliari. 2002. *Ostrinia nubilalis* parasitism and the field abundance of non-target insects in transgenic *Bacillus thuringiensis* corn (*Zea mays*). Environ. Biosafety Res. 1:49–60.

Bourguet, D., J. Chaufaux, M. Se´quin, C. Buisson, J.L. Hinton, T.J. Stodola, P. Porter, G. Cronholm, L. Buschman, and D.A. Andow. 2003. Frequency of alleles conferring resistance to Bt maize in French and US corn belt populations of the European corn borer, *Ostrinia nubilalis*. Theor. Appl. Genet. 106:1225-1233.

Broderick, N., F.K. Raffa, and J. Handelsman. 2006. Midgut bacteria required for *Bacillus thruingiensis* insecticidal activity. Proc. Natl. Acad. Sci. (USA) 103:15196-15199.

Brunet, Y., X. Foueillassar, A. Audran, D. Garrigou, S. Dayau, and L. Tardieu. 2003. Evidence for long-range transport of viable maize pollen. Proceedings of the 1st European Conference on the Co-existence of Genetically Modified Crops with Conventional and Organic Crops. Denmark. p 74-76.

Burton, S.L., D.J. Ellar, J. Li, and D.J Derbyshire. 1999. N-acetylgalactosamine on the putative insect receptor aminopeptidase N is recognised by a site on the domain III lectin-like fold of a *Bacillus thuringiensis* insecticidal toxin. J Mol. Biol. 287:1011-1022.

Butko, P., F. Huang, M. Pusztai-Carey, and W.K. Surewicz. 1997. Membrane permeabilization induced by cytolytic δ-endotoxin CytA from *Bacillus thuringiensis* var. *israelensis*. Biochemistry 35:11355-11360.

Candolfi, M.P., K. Brown, C. Grimm, B. Reber, and H. Schmidli. 2004. A faunistic approach to assess potential side-effects of genetically modified Bt-corn on non-target arthropods under field conditions. Biocontrol Sci. Technol. 14:129-170.

Carlton, B.C. and J.M. Gonzalez. 1985. The genetics and molecular biology of *Bacillus thuringiensis*. *In:* Dubrau, D. (Ed.), The Molecular Biology of the Bacilli, Vol II. Academic Press, New York. p 211-249.

Carroll, J., D. Convents, J. Van Damme, A. Boets, J. Van Rie, and D.J. Ellar. 1997. Intramolecular proteolytic cleavage of *Bacillus thuringiensis* Cry3A delta-endotoxin may facilitate its coleopteran toxicity. J. Invertebr. Pathol. 70:41-49.

Cheng, M., B.A. Lowe, T.M. Spencer, X. Ye, and C.L. Armstrong. 2004. Invited reviews: Factors influencing *Agrobacterium*-mediated transformation of monocotyledonous species. *In Vitro* Cell. Dev. Biol. Plant 40:31-45.

Chowdhury, E.H., H. Kuribara, A. Hino, P. Sultana, O. Mikami, N. Shimada, K.S. Guruge, M. Saito, and Y. Nakajima. 2003. Detection of corn intrinsic and recombinant DNA gragments and Cry1AB protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11. J. Anim. Sci. 81:2546-2551.

Clairmont, F.R., R.E. Milne, V.T. Pham, M.B. Carriere, and H. Kaplan. 1998. Role of DNA in the activation of the Cry1A insecticidal crystal protein from *Bacillus thuringiensis*. J. Biol. Chem. 273:9292-9296.

Claus, D. and R.C.W. Berkeley. 1986. Genus *Bacillus* Cohn 1872, 174. *In*: Sneath, P.H.A., N.S. Mair, M.E. Sharpe, and J.G. Holt (Eds.), Bergey's Manual of Systematic Bacteriology, Vol. 2. Williams and Wilkins, Baltimore, MD. p 1104-1139.

Crecchio, C. and G. Stotzky. 2001. Biodegradation and insecticidal activity of the toxin from *Bacillus thuringiensis* subsp. *kurstaki* bound on complexes of montmorillonite-humic acids-Al hydroxypolymers. Soil Biol. Biochem. 33:573-581.

Crickmore, N., D.R. Zeigler, J. Feitelson, E. Schnepf, D. Lereclus, J. Baum, J. Van Rie, and D.H. Dean. 1998. Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. Microbiol. Mol. Biol. Rev. 63:807-813.

Crickmore, N., D.R. Zeigler, E. Schnepf, J. Van Rie, D. Lereclus, J. Baum, A. Bravo, and D.H. Dean. 2005. *Bacillus thuringiensis* Toxin Nomenclature (Homepage). [cited January 2006]. http://www.lifesci.sussex.ac.uk/Home/Neil_Crickmore/Bt/

Cristofoletti, P.T. and W.R. Terra. 2000. The role of amino acid residues in the active site of a midgut microvillar aminopeptidase from the beetle *Tenebrio molitor*. Biochim. Biophys Acta 1479:185-195.

Daly, T. and G.D. Buntin. 2005. Effect of *Bacillus thuringiensis* transgenic corn for lepidopteran control on nontarget arthropods. Environ. Entomol. 34:1292-1301.

Dankocsik, C., W.P. Donovan, and C.S. Jany. 1990. Activation of a cryptic crystal protein gene of *Bacillus thuringiensis* subspecies *kurstaki* by gene fusion and determination of the crystal protein insecticidal specificity. Mol. Microbiol. 4:2087-2094.

De Cosa, B., W. Moar, S.B. Lee, M. Miller, and H. Daniell. 2001. Overexpression of the Bt cry2Aa2 operon in chloroplasts leads to formation of insecticidal crystals. Nat. Biotechnol. 19:71-74.

De la Riva, G. and M.J. Adang. 1996. Expression of *Bacillus thuringiensis* δ-endotoxin genes in transgenic plants. Biotecn. Applic. 13:251-260.

Dean, D.H., F. Rajamohan, M.K. Lee, S.-J. Wu, X.J. Chen, E. Alcantara, and S.R. Hussain. 1996. Probing the mechanism of action of *Bacillus thuringiensis* insecticidal proteins by site-directed mutagenesis - a minireview. Gene 179:111-117.

DEKALB Genetics Corporation. 1997. USEPA Cry1Ac-corn registration submission, MRID (Master Record Identification) #439995 (The MRID numbers provided represent a package of documents submitted to support a registration submission. See Tables and References in Annex for individual reports). *

Del Rincon-Castro, M.C., J. Barajas-Huerta, and J.E. Ibarra. 1999. Antagonism between Cry1Ac1 and Cyt1A1 toxins of *Bacillus thuringiensis*. Appl. Envir. Microbiol. 65:2049-2053.

Delucca, A.J., J.G Simonson, and A.D. Larson. 1981. *Bacillus thuringiensis* distribution in soils of the United States. Can. J. Microbiol. 27:865-870.

Denolf, P., K. Hendrickx, J. Van Damme, S. Jansens, M. Peferoen, D. Degheele, and J. Van Rie. 1997. Cloning and characterization of *Manduca sexta* and *Plutella xylostella* midgut aminopeptidase N enzymes related to *Bacillus thuringiensis* toxin-binding proteins. Eur. J. Biochem. 248:748-761.

Denolf, P., S. Jansens, M. Perfereon, D. Degheele, and J. Van Rie. 1993. Two different *Bacillus thuringiensis* delta-endotoxin receptors in the midgut brush border membrane of the European corn borer *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae). Appl. Environ. Microbiol. 59:1828-1837.

Devare, M.H., C.M. Jones, and J.E. Thies. 2004. Effect of Cry3Bb transgenic corn and tefluthrin on the soil microbial community biomass, activity, and diversity. J. Environ. Qual. 33:837-843.

Dimock, M.B., R.M. Beach, and P.S. Carlson. 1988. Endophytic bacteria for the delivery of crop protection agents. *In*: Roberts, D.W. and R.R. Granados (Eds.), Biotechnology, Biological Pesticides and Novel Plant-Pest Resistance for Insect Pest Management. Boyce Thompson Institute, Ithaca, NY. p 88-92.

Dively, G. P. 2005. Impact of transgenic VIP3A × Cry1Ab lepidopteran-resistant field corn on the nontarget arthropod community. Environ. Entomol. 34:1267-1291.

* Health and safety studies submitted to the US Environmental Protection Agency in support of pesticidal registration can be released to the general public (with a few restrictions) after the product is registered. These, as identified by a Master Record Identification (MRID) number, can be obtained through the Freedom of Information Office at: HQ FOIA Operations Staff, United States Environmental Protection Agency, 1200 Pennsylvania Avenue (1105A), Washington, DC 20460, (202) 564-7333, Email: hq.foia@epa.gov, web page address: http://www.epa.gov/foia/.

Dively, G.P. and R.Rose. 2003. Effects of *Bt* transgenic and conventional insecticide control on the non-target natural enemy community in sweet corn. *In:* Van DriescheR.G. (Ed.), Proceedings of the 1st International Symposium on Biological Control of Arthropods. U.S. Dept. Agric. Forest Service, Morgantown, WV. P 265-274.

Dively, G.P., R. Rose, M.K. Sears, R.L Hellmich, D.E. Stanley-Horn, D.D. Calvin, J.M Russo, and P.L. Anderson. 2004. Effects on monarch butterfly larvae (Lepidoptera: Danaidae) after continuous exposure to Cry1Ab-expressing corn during anthesis. Environ. Entomol. 33:1116-1125

Dogan, E.B., R.E. Berry, G.L. Reed, and P.A Rossignol. 1996. Biological parameters of convergent lady beetle (Coleoptera: Coccinellidae) feeding on aphids (Homoptera: Aphididae) on transgenic potato. J. Econ. Entomol. 89: 1105–1108.

Du, J., B.H. Knowles, J. Li, and D.J. Ellar. 1999. Biochemical characterization of *Bacillus thuringiensis* cytolytic toxins in association with a phospholipid bilayer. Biochem. J. 338:185-193.

Duan, J.J., G. Head, M.J. McKee, T.E. Nickson, J.W. Martin, and F.S. Sayegh. 2002: Evaluation of transgenic corn pollen expressing Cry3Bb1 protein on a non-target ladybird beetle, *Coleomegilla maculata*. Entomol. Exp. Applic. 104:271-280.

Duan, J.J., M.S. Paradise, J.G. Lundgren, J.T. Bookout, C. Jiang, and R.N. Wiedenmann. 2006. Assessing nontarget impacts of Bt corn resistant to corn rootworms: Tier-1 testing with larvae of *Poecilus chalcites* (Coleoptera: Carabidae). Environ. Entomol. 35:135-142.

Dubelman, S., B. Ayden, B. Bader, C. Brown, C. Jiang, and D. Vlachos. 2005. Cry1Ab protein does not persist in soil after 3 years of sustained Bt corn use. Environ. Entomol. 34:915-921.

DuBois, N. 1998. Personal communication.

Duffield, S.J. and Aebischer, N.J. 1994 The effect of spatial scale of treatment with dimethoate on invertebrate population recovery in winter wheat. J. Appl. Ecol. 31:263-281.

Dunfield, K.E. and J.J. Germida. 2004. Impact of genetically modified crops on soil- and plant- associated microbial communities. J. Environ. Qual. 33:806-815.

Dutton, A., M. D'Alessandro, J. Romeis, and F. Bigler. 2004. Assessing expression of Bt-toxin (Cry1Ab) in transgenic maize under different environmental conditions. IOBC/WPRS Bull. 27:49-55.

Dutton, A., H. Klein, J. Romeis, and F. Bigler. 2002. Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. Ecol. Entomol. 27:441-447.

Dutton, A., J. Romeis, and F. Bigler. 2003. Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: Bt-maize expressing Cry1Ab as a case study. BioControl 48:611-636.

EFSA (European Food Safety Authority). 2004. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safety of foods and food ingredients derived from insect-protected genetically modified maize MON 863 and MON 863 x MON 810, for which a request for placing on the market was submitted under Article 4 of the Novel Food Regulation (EC) No 258/97 by Monsanto. (Question No EFSA-Q-2003-121). The EFSA Journal [Internet] 50:1-25. Available from: http://www.efsa.europa.eu/etc/medialib/efsa/science/gmo/gmo_opinions/383.Par.0001.File.dat/opinion_gmo_07_en1.pdf

EFSA. 2005a. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/F/96/05.10) for the placing on the market of insect-tolerant genetically modified maize Bt11, for cultivation, feed and industrial processing, under Part C of Directive 2001/18/EC from Syngenta Seeds. The EFSA Journal [Internet] 213:1-33. Available from: http://www.efsa.europa.eu/etc/medialib/efsa/science/gmo/gmo_opinions/922.Par.0001.File.dat/gmo_opinion_ej213_bt11maize_cultivation_en1.pdf

EFSA. 2005b. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/ES/01/01) for the placing on the market of insect-tolerant genetically modified maize 1507, for import, feed and industrial processing and cultivation, under Part C of

Directive 2001/18/EC from Pioneer Hi-Bred International/Mycogen Seeds. The EFSA Journal [Internet] 181:1-33. Available from: http://www.efsa.europa.eu/etc/medialib/efsa/science/gmo/gmo_opinions/827.Par.0001.File.dat/op_gm08_ej181_1507_opinion_doc1_2en1.pdf.

EFSA. 2005c Opinion of the Scientific Panel on Genetically Modified Organisms on an application (Reference EFSA GMO DE 2004 03) for the placing on the market of insect protected genetically modified maize MON 863 x MON 810, for food and feed use, under Regulation (EC) No 1829/2003 from Monsanto, The EFSA Journal [Internet] 252:1-23. Available from: http://www.efsa.europa.eu/etc/medialib/efsa/science/gmo/gmo_opinions/1031.Par.0001.File.dat/gmo_opinion_ej252_mon863x810_2_en1.pdf

Einspanier, R., B. Lutz, S. Rief, O. Berezina, V. Zverlov, W. Schwarz, and J. Mayer. 2004. Tracing residual recombinant feed molecules during digestion and rumen bacterial diversity in cattle fed transgene maize. Eur. Food Res. Technol. 218:269-273.

Ejiofor, A.O. and T. Johnson. 2002. Physiological and molecular detection of crystalliferous *Bacillus thuringiensis* strains from habitats in the South Central United States. J. Ind. Microbiol. Biotechnol. 28:284-290.

Emberlin, J., B. Adams-Groom, and J. Tidmarsh. 1999. A report on the dispersal of maize pollen. Soil Association. Bristol, UK. Available from: http://www.soilassociation.org/web/sa/saweb.nsf/848d689047cb466780256a6b00298980/80256ad8005545498025672800383801!OpenDocument

Emmerling, M., D. Chandler, and M. Sandeman. 2001. Molecular cloning of three cDMAs encoding aminopeptidases from the midgut of *Helicoverpa punctigera*, the Australian native budworm. Insect Biochem. Mol. Biol. 31:899-907.

Escher, N., B. Käch, and W. Nentwig. 2000. Decomposition of transgenic *Bacillus thuringiensis* maize by microorganisms and woodlice *Porcellio scaber* (Crustacea: Isopoda). Basic Appl. Ecol. 1:161-169.

Eschriche, B., N. de Decker, J. Van Rie, S. Jansens, and E. van Kerkhove. 1998. Changes in permeability of brush border membrane vesicles from *Spodoptera littoralis* midgut induced by insecticidal crystal proteins from *Bacillus thuringiensis.* Appl. Environ. Microbiol. 64:1563-1565.

Escriche, B., J. Ferre, and F.J. Silva. 1997. Occurrence of a common binding site in *Mamestra brassicae*, *Phthorimaea operculella,* and *Spodoptera exigua* for the insecticidal crystal proteins CryIA from *Bacillus thuringiensis*. Insect Biochem. Mol. Biol. 27:651-656.

Escriche, B., A.C. Martinez-Ramirez, M.D. Real, F.J. Silva, and J. Ferré. 1994. Occurrence of three different binding sites for *Bacillus thuringiensis* δ-endotoxins in the midgut brush border membrane of potato tuber moth, *Phthorimaea operculella* (Zeller). Arch. Insect Biochem. Physiol. 26:315-327.

Estada, U. and J. Ferré. 1994. Binding of insecticidal crystal proteins of *Bacillus thuringiensis* to the midgut brush border membrane of the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), and selection for resistance to one of the crystal proteins. Appl. Environ. Microbiol. 60:3840-3846.

Estela, A., B. Escriche, and J. Ferre. 2004. Interaction of *Bacillus thuringiensis* Toxins with Larval Midgut Binding Sites of *Helicoverpa armigera* (Lepidoptera: Noctuidae). Appl. Environ. Microbiol. 70:1378-1384.

Estruch, J.J., S. Kadwell, E. Merlin, and L. Crossland. 1994. Cloning and characterization of a maize pollen-specific calcium-dependent calmodulin-independent protein kinase. Proc. Natl. Acad. Sci. (USA) 91:8837-8841.

Estruch, J.J., G.W. Warren, M.A. Mullins, G.J. Nye, J.A. Craig, and M.G. Koziel. 1996. Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. Proc. Natl. Acad. Sci. (USA) 93:5389-5394.

Evans, H.F. 2002. Environmental Impact of *Bt* Exudates from Roots of Genetically Modified Crops, Final Report. Department for Environment Food and Rural Affairs, London. 130 p. Available from: http://www.defra.gov.uk/environment/gm/research/pdf/epg-1-5-156.htm

Farinós, G.P., M. de la Poza, P. Hernández-Crespo, F. Ortego, and P. Castañera. 2004. Resistance monitoring of field populations of the corn borers Sesamia nonagrioides and Ostrinia nubilalis after 5 years of Bt maize cultivation in Spain. Entomol. Exp. App. 110:23-30.

Federici, B.A. 1993. Insecticidal bacterial proteins identify the midgut epithelium as a source of novel target sites for insect control. Arch. Insect Biochem. Physiol. 22:357-371.

Federici, B.A. 2002. Case study: Bt crops – a novel mode of insect control. *In*: K.T. Atherton (Ed.), Genetically Modified Crops: Assessing Safety. Taylor and Francis, New York. p 164-200.

Feitelson, J.J., J. Payne, and L. Kim. 1992. *Bacillus thuringiensis*: Insects and beyond. Biotechnology 10:271-276.

Felke, M. and G.A. Langenbruch. 2001. Gefährdet *Bt*-Pollen Schmetterlinge? Gesunde Pflanzen 53:24–28.

Felke, M., and G.A. Langenbruch. 2003. Wirkung von Bt-Mais-Pollen auf Raupen des Tagpfauenauges im Laborversuch. Gesunde Pflanzen, 55:1–7.

Felke, M., N. Lorenz, and G.A. Langenbruch. 2002. Laboratory studies on the effects of pollen from Btmaize on larvae of some butterfly species. J. Appl. Entomol. 126:320–325.

Ferrandis, M.D., P.V.M. Jaurez, R.Frutos, Y. Bel, and J. Ferre. 1999. Distribution of cryI, cryII and cryV genes within *Bacillus thuringiensis* isolates from Spain. Syst. Appl. Microbiol. 2:179-185.

Fischoff, D.A., K.S. Bowdish, F.J. Perlak, P.G. Marrone, S.M. McCormick, J.G. Niedermeyer, D.A. Dean, K. Kusano-Kretzmer, E.J. Mayer, D.E. Rochester, S.G. Rogers, and R.T. Fraley. 1987. Insect tolerant transgenic tomato plants. Biotechnology 5:807-813.

Fitt, G.P. and L.J. Wilson. 2002. Non-target effects of Bt-cotton: a case study from Australia. *In:* Akhurst, R.J., C.E Beard, and P.A. Hughes (Eds.), Biotechnology of *Bacillus thuringiensis* and its Environmental Impact. Proceedings of the 4th Pacific Rim Conderence, Canberra, Australia. p 175-182.

Flores, S., D. Saxena, and G. Stotzky. 2005. Transgenic Bt plants decompose less in soil than non-Bt plants. Soil Biol. Biochem. 37:1073-1082.

Fox, J. 2003. Resistance to Bt toxin surprisingly absent from pests. Nat. Biotechnol. 21:958-959.

Francis, B.R. and L.A. Bulla. 1997. Further characterization of BT-R1, the cadherin-like receptor for Cry1Ab toxin in tobacco hornworm (*Manduca sexta*) midguts. Insect Biochem. Mol. Biol. 27:541-550.

Frankenhuyzen, K van. and C. Nystrom. 1999. The *Bacillus thuringiensis* toxin specificity database. Available from: http://www.glfc.cfs.nrcan.gc.ca/science/research/netintro99_e.html

Galitsky, N., V. Cody, A. Wojtczak, D. Ghosh, J.R. Luft, W. Pangborn, and W. English. 2001. Structure of the insecticidal bacterial delta-endotoxin Cry3Bb1 of *Bacillus thuringiensis*. Acta Crystallogr. D. Biol. Crystallogr. 57:1101-1109.

Gahan, L.J., F. Gould, and D.G. Heckel. 2001. Identification of a gene associated with Bt resistance in *Heliothis virescens*. Science 293:857-860.

Garner, K., S. Hiremath, K. Lehtoma, and A. P. Valaitis. 1999. Cloning and complete sequence characterization of two gypsy moth aminopeptidase-N cDNAs, including the receptor for *Bacillus thuringiensis* Cry1Ac toxin. Insect Biochem. Mol. Biol. 29:527-535.

Gasser, C. S. and R.T. Fraley. 1989. Genetically engineering plants for crop imporvement. Science 244:1293-1299.

Gazit, E., N. Burshtein, D.J. Ellar, T. Sawyer, and Y. Shai. 1997. *Bacillus thuringiensis* cytolytic toxin associates specifically with its synthetic helices A and C in the membrane bound state. Implications for the assembly of oligomeric transmembrane pores. Biochemistry 36:15546-15554.

Gerber, D. and Y. Shai. 2000. Insertion and organization within membranes of the δ-endotoxin pore-forming domain, helix 4-loop-helix 5, and inhibition of its activity by a mutant helix 4 peptide. J. Biol. Chem. 275:23602-23607.

Gill, M. and D. Ellar. 2002. Transgenic Drosophilia reveals a fucntional in vivio receptor for the *Bacillus thuringiensis* toxin Cry1Ac1. Insect Mol. Biol. 11:619-625.

Gilliland, A., C.E. Chambers, E.J. Bone, and D.J. Ellar. 2002. Role of *Bacillus thuringiensis* Cry1 δ-endotoxin binding in determining potency during lepidopteran larval development. Appl. Envior. Microbiol. 68:1509-1515.

Glare, T.R. and M. O'Callaghan. 2000. *Bacillus thuringiensis:* Biology, Ecology and Safety. John Wiley and Sons, Ltd., West Sussex, UK.

GLP (Good Laboratory Practice). 2006. Organisation for Economic Co-operation and Development, Paris. Available from: http://www.oecd.org/department/0,2688,en_2649_34381_1_1_1_1_1,00.html

Gould, F., A. Martinez-Ramirez, A. Anderson, J. Ferré, F.J. Silva, and W.J. Moar. 1992. Broad-spectrum resistance to *Bacillus thuringiensis* toxins in *Heliothis virescens*. Proc. Natl. Acad. Sci. (USA) 89:7986-7990.

Greenplate, J. 1997. Response to reports of early damage in 1996 commercial Bt transgenic cotton (Bollgard™) plantings. Soc. Invertebr. Pathol. Newslett. 29:15-18.

Greenplate, J.T. 1999. Quantification of *Bacillus thuringiensis* insect control protein Cry1Ac over time in Bollgard cotton fruit and terminals. J. Econ. Entomol. 92:1377-1383.

Griffitts, J.S., S.M. Haslam, T. Yang, S.F. Garczynski, B. Mulloy, H. Morris, P.C. Cremer, A.Dell, M.J. Adang, and R.V. Aroian. 2005. Glycolipids as receptor for *Bacillus thuringiensis* Crystal toxin. Science 307:922–925

Griffitts, J.S., J.L. Whitacre, D.E. Stevens, and R.V.Aroian. 2001. Bt toxin resistance from loss of a putative carbohydrate-modifying enzyme. Science 293:860-864

Grochulski, P., L. Masson, S. Borisova, M. Pusztai-Carey, J.L. Schwartz, R. Broussear, and M. Cygler. 1995. *Bacillus thuringiensis* CryIA(a) insecticidal toxin: crystal structure and channel formation. J. Mol. Biol. 254:447-464.

Guo, J.Y., F.H. Wan, and L. Dong. 2004. Survival and developmet of immature *Chrysopa sinica* and *Propylaea japonica* feeding on Bemisia tabaci propagated on transgenic Bt cotton. Chin. J. Biol. Control 20:164-169.

Halfhill, M.D., R.J. Millwood, P.L.Raymer, and C.N. Stewart, Jr. 2002. Bt-transgenic oilseed rape hybridization with its weedy relative, Brassica rapa. Environ. Biosafety Res. 1:19-28.

Hansen Jesse, L.C. and J.J. Obrycki. 2000. Field deposition of Bt transgenic corn pollen: lethal effects on the monarch butterfly. Oecologia 125:241–248.

Hansen Jesse, L.C. and J.J. Obrycki. 2002. Assessment of the non-target effects of transgenic Bt corn pollen and anthers on the milkweed tiger moth, Euchatias egle Drury (Lepidoptera: Arctiidae). J. Kansas Entomol. Soc. 75:55-58.

Harwood, J.D. and J.J. Obrycki. 2006. The detection and decay of Cry1Ab Bt-endotoxins within non-target slugs, *Deroceras reticulatum* (Mollusca: Pulmonata), following consumption of transgenic corn. Biocontrol Sci. Technol. 16:77-88.

Harwood, J.D., W.G. Wallin, and J.J. Obrycki. 2005. Uptake of Bt endotoxins by non-target herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem. Mol. Ecol. 14:2815-2823.

Head, G., C.R. Brown, M.E. Groth, J.J., and Duan. 2001. Cry1Ab protein levels in phytophagous insects feeding on transgenic corn: implications for secondary exposure risk assessment. Entomol. Exp. Appl. 99:37-45.

Head, G., W. Moar, M. Eubanks, B. Freeman, J. Ruberson, A. Hagerty, and S. Turnipseed. 2005. A Multiyear, Large-Scale Comparison of Arthropod Populations on Commercially Managed Bt and Non-Bt Cotton Fields. Environ. Entomol. 34:1257-1266.

Head, G., J.B. Surber, J.A. Watson, J.W. Martin, and J.J. Duan. 2002. No detection of Cry1Ac protein in soil after multiple years of transgenic Bt cotton (Bollguard) use. Environ. Entomol. 31:30-36.

Hellmich, R.L., B.D. Siegfried, M.K. Sears, D.E. Stanley-Horn, M. J. Daniels, H.R. Mattila, T.Spencer, K.G. Bidne, and L.C. Lewis. 2001. Monarch larvae sensitivity to *Bacillus thuringiensis*-purified proteins and pollen. Proc. Natl. Acad. Sci. (USA) 98:11925-11930.

Hendriksen, N.B. and B.M. Hansen. 2002. Long-term survival and germination of *Bacillus thuringiensis* var. *kurstaki* in a field trial. Can. J. Microbiol. 48:256-261.

Hendriksen, N.B. and B.M. Hansen. 2004. Unpublished research. Personal communication.

Herman, R.A., S.L. Evans, D.M. Shanahan, C.A. Mihaliak, G.A. Bormett, D.L. Yound, and J. Buehrer. 2001. Rapid degradation of Cry1F delta-endotoxin in soil. Environ. Entomol. 30:642-644.

Herman, R.A., B.W. Schafer, B.A. Korjagin, and A.D. Ernest. 2003. Rapid digestion of Cry34Ab1 and Cry35Ab1 in simulated grastric fluid. J. Agric. Food Chem. 51:6823-6827.

Herman, R.A., P.N. Scherer, and J.D. Wolt. 2002a. Rapid degradation of binary, PS149B1, delta-endotoxin of *Bacillus thuringiensis* in soil, and a novel mathematical model for fitting curve-linear decay. Environ. Entomol. 31:208-214.

Herman, R.A., J.D. Wolt, and W.R. Halliday. 2002b. Rapid degradation of the Cry1F insecticidal crystal protein in soil. J. Agric. Food Chem. 50:7076-7078.

Hernandez, C.S., J. Ferre, and I Larget-Thiery. 2001. Update on the detection of beta-exotoxin in *Bacillus thurnigiensis* by HPLC analysis. J. Appl. Microbiol. 90:643-647.

Hernandez, E., F. Ramisse, T. Cruel, R. leVagueresse, and J.D. Cavallo. 1999. *Bacillus thuringiensis s*erotype H34 isolated from human and insecticidal strains serotypes 3a3b and H14 can lead to death of immunocompetent mice after pulmonary infection. FEMS Immunol. Med. Microbiol. 24:43-47.

Hernandez, E., F. Ramisse, J.P. Ducoureau, T. Cruel, and J.D. Cavallo. 1998. *Bacillus thuringiensis* subsp. *konkukian* (Serotype H34) Superinfection: Case Report and Experimental Evidence of Pathogenicity in Immunosupressed Mice. J. Clin. Microbiol. 36:2138-2139.

Hernandez, E., F. Ramisse, P. Gros, and J.D. Cavallo. 2000. Super-infection by *Bacillus thuringiensis* H34 or 3a3b can lead to death in mice infected with the influenza A virus. FEMS Immunol. Med. Microbiol. 29:177-181.

Hilbeck, A. 2002. Transgenic host plant resistance and non-target effects *In*: Letournear, D.K. and B.E. Burrows (Eds.), Genetically Engineered Organisms: Assessing Environmental and Human Health Effects. CRC Press, Boca Raton, Fla. p 167-185.

Hilbeck, A., M. Baumgartner, P.M. Fried, and F. Bigler. 1998a. Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea*) (Neuroptera: Chrysopidas). Environ. Entomol. 27:480-487.

Hilbeck, A., W.J. Moar, M. Pusztai-Carey, A. Filippini, and F. Bigler. 1998b. Toxicity of *Bacillus thuringiensis* Cry1Ab Toxin to the Predator *Chrysoperla carnea* (*Neuroptera: Chrysopidae*). Environ. Entomol. 27:1255-1263.

Hilbeck, A, W.J. Moar, M. Pusztai-Carey, A. Filippini, and F. Bigler. 1999. Prey-mediated effects of CryIAb toxin and protoxin and Cry2A protoxin on the predator *Chrysoperla carnea*. Entomol. Exp. Appl. 91:305–316.

Hill, K.K., L.O. Ticknor, R.T. Okinaka, M. Asay, H. Blair, K.A. Bilss, M. Laker, P.E. Pardington, A.P. Richardson, M. Tonks, D.J. Beecher, J.D. Kemp, A-B. Kolsto, A.C.L. Wong, P. Keim, and P.J. Jackson. 2004 Fluorescent amplified fragment length polymorphism analysis of Bacillus anthracis, Bacillus cereus, and Bacillus thuringiensis isolates. Appl. Environ. Microbiol. 70:1068-1080.

Hoffmann, C., H.Vanderbruggen, H.Hofte, J. Van Rie, S. Jansens, and H. Van Mellaert. 1988. Specificity of Bacillus thuringiensis δ-endotoxins is correlated with the presence of high-affinity binding sites in the brush border membrane of target insect midguts. Proc. Natl. Acad. Sci. (USA) 85:7844-7848.

Hofte, H. and H.R. Whiteley. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. Microbiol. Rev. 53:242-255.

Hopkins, D.W. and E.G. Gregorich. 2005. Decomposition of residues and loss of the δ-endotoxin from transgenic (Bt) corn (Zea mays L.) in soil. Can. J. Soil Sci. 85:19-26.

Howald, R., C. Zwahlen, and W. Nentwig. 2003. Evaluation of Bt oilseed rape on the non-target herbivore *Athalia rosae*. Entomol. Exp. Appl. 106:87-93.

Hua, G., L. Masson, J.L. Jurat-Fuentes, G. Schwab, and M.J. Adang. 2001. Binding Analyses of *Bacillus thuringiensis* Cry δ-endotoxins Using Brush Border Membrane Vesicles of *Ostrinia nubilalis*. Appl. Environ. Microbiol. 67:872-879.

Hua, G., K. Tsukamoto, M.L. Rasilo, and H. Ikezawa.1998. Molecular cloning of a BPI-anchored aminopeptidase N from *Bombyx mori* midgut: a putative receptor for *Bacillus thuringiensis* CryIA toxin. Gene 214:177-185.

Huang, F., L.L. Buschman, R.A. Higgins, and H. Li. 2002. Survival of Kansas Dipel-resistant European corn borer (Lepidoptera: Crambidae) on Bt and non-Bt corn hybrids. J. Econ. Entomol. 95:614-621.

Hudspeth, R.L. and J.W. Grula. 1989. Structure and expression of the maize gene encoding the phosphoenolpyruvate carboxylase isozyme involved in C4 photosynthesis. Plant Mol. Biol. 12:579-589.

Huffman, D.L., L.J. Bischof, J.S. Griffitts, and R.V. Aroian. 2004. Pore worms: Using *Caenorhabditis elegans* to study how bacterial toxins interact with their target host. Int. J. Med. Microbiol. 293:599-607.

Ishiwata, S. 1901. On a kind of severe flacherie (sotto disease) (No. 1). Dainihon Sanshi Kaiho 114:1-5.

Jasinski, J.R., J.B. Eisley, C.E. Young, J. Kovach, and H. Willson. 2003. Select non-target arthropod abundance in transgenic and non-transgenic field crops in Ohio. Environ. Entomol. 32: 407-413.

Jenkins, J.L., M.K. Lee, S. Sangadala, M.J. Adang, and D.H. Dean. 1999. Binding of *Bacillus thuringiensis* Cry1Ac toxin to *Manduca sexta* aminopeptidase-N receptor is not directly related to toxicity. FEBS Lett. 462:373-376.

Jenkins, J.L., M.K. Lee, A.P. Valaitis, A. Curtiss, and D.H. Dean. 2000. Bivalent sequential binding model of a *Bacillus thuringiensis* toxin to gypsy moth aminopeptidase N receptor. J. Biol. Chem. 275:14423-14431.

Jennings, J.C., L.D. Albee, D.C. Kolwyck, J.B. Surber, M.L. Taylor, G.F. Hartnell, R.P. Lirette, and K.C. Glenn. 2003. Attempts to detect transgenic and endogenous plant DNA and transgenic protein in muscle from broilers fed YieldGard Corn Borer corn. Poult. Sci. 82:371-380.

Jesse, L.C.H. and J.J. Obrycki. 2003. Occurrence of *Danaus plexippus* L. (Lepidoptera: Danaidae) on milkweeds (*Asclepias syriaca*) in transgenic Bt corn agroecosystems. Agric. Ecosyst. Environ. 97:225-233.

Johnson, K.S., J.M. Scriber, J.K. Nitao, and D.R. Smitley. 1995. Toxicity of var. *kurstaki* to three non-target Lepidoptera in field studies. Environ. Entomol. 24:288-297.

Jurat-Fuentes, J.L., F.L. Gould, and M.J. Adang. 2000. High levels of resistance and cross-resistance to *Bacillus thuringiensis* Cry1 toxins in *Heliothis virescens* are due to reduced toxin binding and pore formation. Resist.Pest Manag. Newslett. 11:23-24.

Kalushkov, P. and O. Nedved. 2005. Genetcially modified potatoes expressing Cry3A protein do not affect aphidophagous coccinellids. J. Appl. Entomol. 129:401-406.

Keeton, T.P. and L.A. Bulla, Jr. 1997. Ligand Specificity and Affinity of BT-R1, the *Bacillus thuringiensis* Cry1A Toxin Receptor from *Manduca Sexta*, Expressed in Mammalian and Insect Cell Cultures. Appl. Environ. Microbiol. 63:3419-3425.

Keim, P., A. Kalif, J. Schupp, K. Hill, S.E. Travis, K. Richmond, D.M. Adair, M. Hugh-Jones, S.R. Kuske, and P.J. Jackson. 1997. Molecular evolution and diversity in *Bacillus anthracis* as detected by amplified fragment length polymorphism markers. J. Bacteriol. 179:818-824.

Kim, H.S., D.W. Lee, S.D. Woo, and Y.M. Yu. 1998. Distribution, serological identification, and PCR analysis of *Bacillus thuringiensis* isolated from soils of Korea. Curr. Microbiol. 37:195-200.

Klier, A., C. Bourgouin, and G. Rapapori. 1983. Mating between *Bacillus subtilitis* and *Bacillus thuringiensis* and transfer of cloned crystal genes. Mol. Gen. Genet. 191:257-262.

Knight, P.J., N. Crickmore, and D.J. Ellar. 1994. The receptor for *Bacillus thuringiensis* CryIA(c) delta-endotoxin in the brush border membrane of the lepidopteran *Manduca sexta* is aminopeptidase N. Mol. Microbiol. 11:429-436.

Knight, P.J., B.H. Knowles, and D.J. Ellar. 1995. Molecular cloning of an insect aminopeptidase N that serves as a receptor for *Bacillus thuringiensis* CryIA(c) toxin. J. Biol. Chem. 270:17765-17770.

Knowles, B.H. 1994. Mechanism of action of *Bacillus thuringiensis* insecticidal δ-endotoxins. Adv. Insect Physiol. 24:273-308.

Koller, C.N., L.S. Bauer, and R.M. Hollingworth. 1992. Characterization of the pH-mediated solubility of *Bacillus thuringiensis* var. *san diego* native delta-endotoxin crystals. Biochem. Biophys. Res. Comm. 184:692-699.

Kondo, S., M. Ohba, and T. Ishii. 1992. Larvidical activity of *Bacillus thuringiensis* serovar *israelensis* against nuisance chironomid midges (*Dipetera: Chironomidae*) of Japan. Lett. Appl. Microbiol. 15:207-209.

Koni, P.A. and D.J. Ellar. 1994. Biochemical characterization of *Bacillus thuringiensis* cytolytic δ-endotoxins. Microbiology 140:1869-1880.

Koziel, G.M., G.L. Beland, C. Bowman, N.B. Carozzi, R. Crenshaw, L. Crossland, J. Dawson, N. Desai, M. Hill, S. Kadwell, K. Launis, D. Maddox, K. McPherson, M. Heghji, E. Merlin, R. Rhodes, G. Warren, M. Wright, and S. Evola. 1993. Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. Biotechnology 11:194-200.

Kranthi, K.R., S. Naidu, C.S. Dhawad, A. Tatwawadi, K. Mate, E. Patil, A.A. Bharose, G.T. Behere, R.M. Wadaskar, and S. Kranthi . 2005. Temporal and intra-plant variability of Cry1Ac expression in *Bt*-cotton and its influence on the survival of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Noctuidae: Lepidoptera). Curr. Sci. 2:291-298.

Kumar, P.A., P.P Sharma, and V.S. Malik. 1996. The insecticidal proteins of *Bacillus thuringiensis*. Adv. Appl. Microbiol. 42:1-43.

Kwa, M.S., R.A. de Maagd, W.J. Stiekema, J.M. Vlak, and D. Bosch. 1998. Toxicity and binding properties of the *Bacillus thuringiensis* δ-endotoxin Cry1C to cultured insect cells. J. Invertebr. Pathol. 71:121-127.

Lambert, B., H. Hofte, K. Annys, S. Jansens, P. Soetaert, and M. Peferoen. 1992. Novel *Bacillus thuringiensis* insecticidal crystal protein with a silent activity against coleopteran larvae. Appl. Environ. Microbiol. 58:2536-2542.

Lang, A. 2004. Monitoring the impact of Bt maize on butterflies in the field: estimation of required sample sizes. Environ. Biosafety Res. 3:55-66.

Lang, A., C. Ludy, and E. Vojtech. 2004. Dispersion and distribution of *Bt* maize pollen in field margins. J. Plant Dis. Protect. 111:417–428.

Lee, D.H., I.H. Cha, D.S. Woo, and M. Ohba. 2003. Microbial ecology of *Bacillus thuringiensis*: fecal populations recovered from wildlife in Korea. Can. J. Microbiol. 49:465-471.

Lee, M.K., T.H. You, F.L. Gould, and D.H. Dean. 1999. Identification of residues in domain III of *Bacillus thuringiensis* Cry1Ac toxin that affect binding and toxicity. Appl. Environ. Microbiol. 65:4513-4520.

Letourneau, D.K., Robinson, G.S. and Hagen, J.A. 2003. Bt crops: predicting effects of escaped transgenes on the fitness of wild plants and their herbivores. Environ. Biosafety Res. 2:219-246.

Li, H., J. Gonzalez-Cabrera, B. Oppert, J. Ferre, R.A. Higgins, L.L. Bushman, G.A. Radke, K.Y. Zhu, and F. Huang. 2004. Binding analyses of Cry1Ab and Cry1Ac with membrane vesicles from *Bacillus thuringiensis*-resistant and –susceptible *Ostrinia nubilalis*. Biochem. Biophys. Research Comm. 323:52-57.

Li, J. 1996. Insecticidal δ-endotoxins from *Bacillus thuringiensis*. *In*: Parker, M.W. (Ed.), Protein Toxin Structure. R.G. Landes Co., Austin, TX. p 49-78.

Li, J., J. Carroll, and D.J. Ellar. 1991. Crystal structure of insecticidal delta-endotoxin from *Bacillus thuringiensis* at 2.5 A resolution. Nature 353:815-821.

Li, J., D.J. Derbyshire, B. Promdonkoy, and D.J. Ellar. 2001. Structural implications for the transformation of the *Bacillus thuringiensis* delta-endotoxins from water-soluble to membrane-inserted forms. Biochem. Soc. Trans 29: 571-7.

Li, J., P.A. Koni, and D.J. Ellar. 1996. Structure of the mosquitocidal delta-endotoxin from *Bacillus thuringiensis* ssp. *kyushuensis* and implications for membrane pore formation. J. Mol. Biol. 257:129-152.

Li, W., K. Wu, X. Wang, G. Wang, and Y. Guo. 2005. Impact of pollen grains of Bt transgenic corn on the growth and and development of chinese Tussah Silkworm, *Anteraea pernyi* (Lepidoptera: Saturniidae). Environ. Entomol. 34:922-928.

Lightwood, D.J., D.J. Ellar, and P. Jarrett. 2000. Role of proteolysis in determining potency of *Bacillus thuringiensis* Cry1Ac delta-endotoxin. Appl. Environ. Microb. 66:5174-5181.

Liu, W.X., F.H. Wan, and J. Guo. 2002a. Structure and seasonal dynamics of arthropods in transgenic Bt cotton field. Acta Ecol. Sin. 22:729-735.

Liu, W.X., F.H. Wan, J.Y. Guo, and G.L. Lövei. 2002b. Spiders and their seasonal dynamics in transgenic Bt- vs. conventionally managed cotton fields in north-central China. *In*: Samu, F. and Szinetár, C. (Eds.), European Arachnology 2002. Plant Protection Institute and Berzsenyi College, Budapest. p 337-342.

Liu, X.D., B.P. Zhai, X.X. Zhang, and J.M. Zong, 2005a. Impact of transgenic cotton plants on a non-target pest, *Aphis gossypii* Glover. Ecol. Entomol. 30:307-315.

Liu, X.-X., Q.-W. Zhang, J.-Z. Zhao, J.-C. Li, B.-I. Xu, and X.-M. Ma. 2005b. Effects of Bt transgenic cotton lines on the cotton bollworm parasitoid *Microplitis mediator* in the laboratory. Biol. Control 35:134-141.

Liu, Y.B., B.E. Tabashnik, W.J. Moar, and R.A. Smith. 1998. Synergism between *Bacillus thuringiensis* spores and toxins against resistant and susceptible diamonback moths (*Putella xylostella*). Appl. Environ. Microbiol. 64:1385-1389.

Lopez, M.D., J.R. Prasifka, D. Bruck, and L.C. Lewis. 2005. Utility of ground beetle species in field tests of potential non-target effects of Bt crops. Environ. Entomol. 34:1317-1324.

Losey, J.E., R.A. Hufbauer, and R.G. Hartzler. 2003. Enumerating lepidopteran species associated with maize as a first step in risk assessment in the USA. Environ. Biosafety Res. 2:247-261.

Losey, J.E., L.S. Raynor, and M.E. Carter. 1999. Transgenic pollen harms monarch larvae. Nature 399:214.

Lövei, G.L. and S. Arpaia. 2005. The impact of transgenic plants on natural enemies: a critical review of laboratory studies. Entomol. Exp. Appl. 114:1-14.

Lumbierres, B., R. Albajes, and X. Pons. 2004. Transgenic Bt maize and *Rhopalosiphum padi* (Hom., Aphididae) performance. Ecol. Entomol. 29:309-317.

Lundgren, J.C. and R.N. Wiedenmann. 2002. Coleopteran-specific Cry3Bb toxin from transgenic corn pollen does not affect the fitness of a non-target species, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae). Environ. Entomol. 31:1213-1218.

Luo, K., Y.J. Lu, and M.J. Adang. 1996. A 106 kDa form of aminopeptidase is a receptor for *Bacillus thuringiensis* Cry1C delta-endotoxin in the brush border membrane of *Manduca sexta.* Insect Biochem. Mol. Biol. 26:783-791.

Luo, K., S. Sangadala, L. Masson, A. Mazza, R. Brousseau, and M.J. Adang. 1997. The *Heliothis virescens* 170 kDa aminopeptidase functions as "receptor A" by mediating specific *Bacillus thuringiensis* Cry1A δ-endotoxin binding and pore formation. Insect Biochem. Mol. Biol. 27:735-743.

Lutz, B., S. Wiedemann, R. Einspanier, J. Mayer, and C. Albrecht. 2005. Degradation of Cry1Ab protein from genetically modified maize in the bovine gastrointestinal tract. J. Agric. Food Chem. 53:1453-1456.

Maagd, R.A. de, A. Bravo, and N. Crickmore. 2001. How *Bacillus thuringiensis* has evolved specific toxins to colonize the insect world. Trends Genet. 17:193-199.

Maagd, R.A. de, M. Weemen-Hendriks, W. Stiekema, and D. Bosch. 2000. *Bacillus thuringiensis* delta-endotoxin Cry1C domain III can function as a specificity determinant for *Spodoptera exigua* in different, but not all, Cry1-cry1C hybrids. Appl. Environ. Microbiol. 66:1559-1563.

Manceva, S.D., M. Pusztai-Carey, P.S. Russo, and P. Burko. 2005. A detergent-like mechanism of action of the cytolytic toxin Cyt1A from *Bacillus thuringiensis* var. *israelensis*. Biochemistry 44:589-597.

Martin, P.A. and R.S. Travers. 1989. Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. App. Environ. Microbiol. 55:2437-2442.

Marvier, M. 2002. Improving risk assessment for non-target safety of transgenic crops. Ecol. Appl. 12:1119-1124.

Masson, L., B.E. Tabashnik, Y.B. Liu, R. Brosseau, and J.L. Schwartz. 1999. Helix 4 of the *Bacillus thuringiensis* Cry1Aa toxin lines the lumen of the ion channel. J. Biol. Chem. 274:31996-32000.

Matten, S.R. 1998. EPA regulation of plant-pesticides and Bt plant-pesticide resistance management. *In*: Hardy, R., J.B. Segelken, and M. Voionmaa (Eds.), NABC Report 10: Agricultural Biotechnology and Environmental Quality. National Agricultural Biotechnology Council, Ithaca, NY. p 121-141.

Mattila, H.R., M.K. Sears, and J.J. Duan. 2005. Response of *Danaus plexippus* to pollen of two new Bt corn events via laboratory bioassay. Entomol. Exp. Appl. 116:31-41.

McBride, K.E., Z. Svab, D.J. Schaef, P.S. Hogan, D. M. Stalker, and P. Maliga. 1995. Amplification of a chimeric *Bacillus* gene in chloroplasts leads to an extraordinary level of insecticidal crystal protein in tobacco. Biotechnology 13:362-365.

McClintock, J.T., C.R. Schaffer, J.L. Kough, and R.D. Sjoblad. 1995a. Relevant taxonomic considerations for regulation of *Bacillus thuringiensis*-based pesticides by the U.S. Environmental Protection Agency. *In*: Feng, T.-Y. (Ed.), *Bacillus thuringiensis* Biotechnology and Environmental Benefits. Hua Shiang Yuan Publishing Co., Taipei, Taiwan. p 313-325.

McClintock, J.T., C.R. Schaffer, and R.D. Sjoblad. 1995b. A comparative review of the mammalian toxicity of *Bacillus thuringiensis*-based pesticides. Pestic. Sci. 45:95-105.

Meier, M.S. and A. Hilbeck. 2001. Influence of transgenic *Bacillus thuringiensis* corn-fed prey on prey preference of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). Basic Appl. Ecol. 2:35-44.

Meissle, M. E. Vojtech, and G.M. Poppy. 2005. Effects of Bt maize-fed prey on the generalist predator *Poecilus cupreus* L. (Coleoptera: Carabidae). Transgenic Res. 14:123-132.

Men, X., F. Ge, C.A. Edwards, and E.N. Yardim. 2004. Influence of pesticide applications on pest and predatory associated with transgenic Bt cotton and non-transgenic cotton plants. Phytoparasitca 32:246-254.

Men, X., F. Ge, X. Liu, and E.N. Yardim. 2003. Diversity of arthrpopod communities in transgenic Bt cotton and non-transgenic cotton agroecosystems. Environ. Entomol. 32:270-275.

Mendelsohn, M., J. Kough, Z. Vaituzis, and K. Mathews. 2003. Are Bt crops safe? Nat. Biotechnol. 21:1003-1009.

Miller, J.C. 1990. Field assessment of the affects of a microbial pest control agent on non-target Lepidoptera. Am. Entomol. 36:135-139.

Mizuki, E., M. Maeda, R. Tanaka, D.E. Lee, M. Hara, T. Akao, S. Yamashita, H.S. Kim, T. Ichimatsu, and M. Ohba. 2001. *Bacillus thuringiensis*: a common member of microflora in activated sludges of a sewage treatment plant. Curr. Microbiol. 42:422-425.

Monsanto Company. 1995a. USEPA Cry3A-potato registration submission, MRID# 429322 (The MRID numbers provided represent a package of documents submitted to support a registration submission. See Tables and References in Annex for individual reports) *

Monsanto Company. 1995b. USEPA Cry1Ac-cotton registration submission, MRID# 431452.*

Monsanto Company. 1995c. USEPA Cry1Ab-corn registration submission, MRID# 436655.*

Monsanto Company. 1995d. USEPA Cry1Ab-corn registration submission, MRID# 436960.*

* Health and safety studies submitted to the US Environmental Protection Agency in support of pesticidal registration can be released to the general public (with a few restrictions) after the product is registered. These, as identified by a Master Record Identification (MRID) number, can be obtained through the Freedom of Information Office at: HQ FOIA Operations Staff, United States Environmental Protection Agency, 1200 Pennsylvania Avenue (1105A), Washington, DC 20460, (202) 564-7333, Email: hq.foia@epa.gov, web page address: http://www.epa.gov/foia/.

Monsanto Company. 1995e. USEPA Cry3A-potato registration submission, MRID# 441247.*

Monsanto Company. 2001a. USEPA Cry2Ab2-cotton registration submission, MRID# 442353.*

Monsanto Company. 2001b. USEPA Cry2Ab2-cotton registration submission, MRID# 449666.*

Monsanto Company. 2001c. USEPA Cry2Ab2-cotton registration submission, MRID# 450863.*

Monsanto Company. 2001d. USEPA Cry2Ab2-cotton registration submission, MRID# 4553371.*

Monsanto Company. 2002a. USEPA Cry3Bb1-corn registration submission, MRID# 449043.*

Monsanto Company. 2002b. USEPA Cry3Bb1-corn registration submission, MRID# 453613.*

Monsanto Company. 2002c. USEPA Cry3Bb1-corn registration submission, MRID# 454240.*

Monsanto Company. 2002d. USEPA Cry3Bb1-corn registration submission, MRID# 455382.*

Monsanto Company. 2002e. USEPA Cry3Bb1-corn registration submission, MRID# 455770.*

Monsanto Company. 2002f. USEPA Cry3Bb1-corn registration submission, MRID# 457571.*

Monsanto Company. 2002g. USEPA Cry3Bb1-corn registration submission, MRID# 457916. *

Monsanto Company and Novartis Seeds, Inc. 1996a. USEPA Cry1Ab-corn registration submission, MRID# 434680.*

Monsanto Company and Novartis Seeds, Inc. 1996b. USEPA Cry1Ab-corn registration submission, MRID# 434392.*

Monsanto Company and Novartis Seeds, Inc. 1996c. USEPA Cry1Ab-corn registration submission, MRID# 435332.*

Monsanto Company and Novartis Seeds, Inc. 1996d. USEPA Cry1Ab-corn registration submission, MRID# 438879.*

Morse, R.J., T. Yamamoto, and R.M. Stroud. 2001. Structure of Cry2Aa suggests an unexpected receptor binding epitope. Structure 9:409-417.

Motavalli, P.P., R.J. Kremer, M. Fang, and N.E. Means. 2004. Impact of genetically modified crops and their management on soil microbially mediated plant nutrient transformations. J. Environ. Qual. 33:816-824.

Mullin, C.A., M.C. Saunders II, T.W. Leslie, D.J. Biddinger, and S.J. Fleischer. 2005. Toxic and Behavioral Effects to Carabidae of Seed Treatments Used on Cry3Bb1- and Cry1Ab/c-Protected Corn. Environ. Entomol. 34:1626-1636.

Musser, F.R. and A.M. Shelton. 2003. Bt sweet corn and selective insecticides: Impact on pests and predators. J. Econ. Entomol. 96:71-80.

Mycogen Plant Sciences and Novartis Seeds, Inc. 1995a. USEPA Cry1Ab-corn registration submission, MRID# 433236 (The MRID numbers provided represent a package of documents submitted to support a registration submission. See Tables and References in Annex for individual reports)

Mycogen Plant Sciences and Novartis Seeds, Inc. 1995b. USEPA Cry1Ab-corn registration submission, MRID# 433396.*

Mycogen Plant Sciences and Novartis Seeds, Inc. 1995c. USEPA Cry1Ab-corn registration submission, MRID# 434157.*

* Health and safety studies submitted to the US Environmental Protection Agency in support of pesticidal registration can be released to the general public (with a few restrictions) after the product is registered. These, as identified by a Master Record Identification (MRID) number, can be obtained through the Freedom of Information Office at: HQ FOIA Operations Staff, United States Environmental Protection Agency, 1200 Pennsylvania Avenue (1105A), Washington, DC 20460, (202) 564-7333, Email: hq.foia@epa.gov, web page address: http://www.epa.gov/foia/.

Mycogen Plant Sciences and Novartis Seeds, Inc. 1995d. USEPA Cry1Ab-corn registration submission, MRID# 434635.*

Mycogen Seeds and Pioneer Hi-Bred International, Inc. 2001a. USEPA Cry1F-corn registration submission, MRID# 450415.*

Mycogen Seeds and Pioneer Hi-Bred International, Inc. 2001b. USEPA Cry1F-corn registration submission, MRID# 451311.*

Mycogen Seeds and Pioneer Hi-Bred International, Inc. 2001c. USEPA Cry1F-corn registration submission, MRID# 453078.*

Mycogen Seeds and Pioneer Hi-Bred International, Inc. 2001d. USEPA Cry1F-corn registration submission, MRID# 446911.*

Mycogen Seeds and Pioneer Hi-Bred International, Inc. 2001e. USEPA Cry1F-corn registration submission, MRID# 450201.*

Mycogen Seeds and Pioneer Hi-Bred International, Inc. 2001f. USEPA Cry1F-corn registration submission, MRID# 449717.*

Mycogen Seeds and Pioneer Hi-Bred International, Inc. 2001g. USEPA Cry1F-corn registration submission, MRID# 452748.*

Mycogen Seeds and Pioneer Hi-Bred International, Inc. 2001h. USEPA Cry1F-corn registration submission, MRID# 447149.*

Mycogen Seeds and Pioneer Hi-Bred International, Inc. 2005a. USEPA Cry34Ab1/Cry35Ab1-corn registration submission, MRID#452422-07.*

Mycogen Seeds and Pioneer Hi-Bred International, Inc. 2005b. USEPA Cry34Ab1/Cry35Ab1-corn registration submission, MRID#453584-01.*

Mycogen Seeds and Pioneer Hi-Bred International, Inc. 2005c. USEPA Cry34Ab1/Cry35Ab1-corn registration submission, MRID#455845-01.*

Mycogen Seeds and Pioneer Hi-Bred International, Inc. 2005d. USEPA Cry34Ab1/Cry35Ab1-corn registration submission, MRID#458086-01.*

Mycogen Seeds and Pioneer Hi-Bred International, Inc. 2005e. USEPA Cry34Ab1/Cry35Ab1-corn registration submission, MRID#458602-01.*

Mycogen Seeds and Pioneer Hi-Bred International, Inc. 2005f. USEPA Cry34Ab1/Cry35Ab1-corn registration submission, MRID#461239-11.*

Mycogen Seeds and Pioneer Hi-Bred International, Inc. 2005g. USEPA Cry34Ab1/Cry35Ab1-corn registration submission, MRID#457904-03.*

Mycogen Seeds and Pioneer Hi-Bred International, Inc. 2005h. USEPA Cry34Ab1/Cry35Ab1-corn registration submission, MRID#453602-01.*

Mycogen Seeds and Pioneer Hi-Bred International, Inc. 2005i. USEPA Cry34Ab1/Cry35Ab1-corn registration submission, MRID#453407-01.

* Health and safety studies submitted to the US Environmental Protection Agency in support of pesticidal registration can be released to the general public (with a few restrictions) after the product is registered. These, as identified by a Master Record Identification (MRID) number, can be obtained through the Freedom of Information Office at: HQ FOIA Operations Staff, United States Environmental Protection Agency, 1200 Pennsylvania Avenue (1105A), Washington, DC 20460, (202) 564-7333, Email: hq.foia@epa.gov, web page address: http://www.epa.gov/foia/.

Nagamatsu, Y., T. Koike, K. Sasaki, A. Yoshimoto, and Y. Furukawa. 1999. The cadherin-like protein is essential to specificity determination and cytotoxic action of the *Bacillus thuringiensis* insecticidal Cry1Aa toxin. FEBS Lett. 460:385-390.

Nagamatsu, Y., S. Toda, F. Yamaguchi, M. Ogo, M. Kogure, M. Nakamura, Y. Shibata, and T. Katsumoto. 1998. Identification of *Bombyx mori* midgut receptor for *Bacillus thuringiensis* insecticidal CryIAa toxin. Biosci., Biotechnol. Biochem. 62:718-726.

Nakanishi, K., K. Yaoi, N. Shimada, T. Kadotani, and R. Sato. 1999. *Bacillus thuringiensis* insecticidal Cry1Aa toxin binds to a highly conserved region of animopeptidase N in the host insect leading to its evolutionary success. Biochim. Biophys. Acta. 1:57-63.

Nannipieri, P., J. Ascher, M.T. Ceccherini, L. Landi, G. Pietramellara, and G. Renella. 2003. Microbial diversity and soil functions. Eur. J. Soil Sci. 54:655-670.

Naranjo, S.E. 2005a. Long-Term Assessment of the Effects of Transgenic Bt Cotton on the Abundance of Nontarget Arthropod Natural Enemies. Environ. Entomol. 34:1193-1210.

Naranjo, S.E. 2005b. Long-Term Assessment of the Effects of Transgenic Bt Cotton on the Function of the Natural Enemy Community. Environ. Entomol. 34:1211-1223.

Naranjo, S.E., G. Head, and G.P. Dively. 2005. Field studies assessing arthropod nontarget effects in Bt transgenic crops: Introduction. Environ. Entomol. 34:1178-1180.

Neher, D.A. 1999. Soil community composition and ecosystem processes. Comparing agricultural ecosystems with natural ecosystems. Agrofor. Syst. 45:159-185.

Nollet, F., P. Kools, and F. van Roy. 2000. Phylogenetic analysis of the cadherin superfamily allows identification of six major subfamilies besides several solitary members. J. Mol. Biol. 299:551-572.

Noteborn, HPJ, M.E. Bienenmann-Ploum, J.H.J. van den Berg, G.M. Alink, L. Zolla, A. Reynaerts, M. Pensa, and H.A. Kuiper. 1995. Safety assessment of the *Bacillus thuringiensis* insecticidal crystal protein CRYIA(b) expressed in tomato. *In*: Engel, K.-H., G.R. Takeoka, and R. Teranishi (Eds.), Genetically Modified Foods: Safety Issues. American Chemical Society, Washington, DC. p 134-147.

Novartis Seeds, Inc. and Monsanto Company. 1996. USEPA Cry1Ab, Cry1Ac, Cry2A, and Cry3A registration submission, MRID# 439416 (The MRID numbers provided represent a package of documents submitted to support a registration submission. See Tables and References in Annex for individual reports). *

NRC (National Research Council). 2000. Genetically Modified Pest-Protected Plants. Science and Regulation. National Academy Press, Washington DC. 261 p.

NSW Department of Land and Water Conservation. 2001. Water Quality in the Namoi Catchment, 2000-2001. NSW Government, Sydney, Australia. 8 p. Available from: http://www.dnr.nsw.gov.au/water/pdf/namoi_water_quality_2000-01.pdf. 8 pages.

Nunez-Valdez, M.-E., J. Sanchez, L. Lina, L. Guereca, and A. Bravo. 2001. Structural and functional studies of α-helix 5 region from *Bacillus thruingiensis* Cry1Ab δ-endotoxins. Biochim. Biophys. Acta 1546:122-133.

Oberhauser, K.S., M.D. Prysby, H.R. Mattila, D.E. Stanley-Horn, M.K. Sears, G. Dively, E. Olson, J.M. Pleasants, W.F. Lam, and R.L. Hellmich. 2001. Temporal and spatial overlap between monarch larvae and corn pollen. Proc. Natl. Acad. Sci. (USA) 98:11913-11918.

Obrist, L.B., H. Klein, A. Dutton, and F. Bigler. 2005. Effects of Bt maize on *Frankliniella tenuicornis* and exposure of thrips predators to prey-mediated Bt toxin. Entomol. Exp. Appl. 115:409-416.

* Health and safety studies submitted to the US Environmental Protection Agency in support of pesticidal registration can be released to the general public (with a few restrictions) after the product is registered. These, as identified by a Master Record Identification (MRID) number, can be obtained through the Freedom of Information Office at: HQ FOIA Operations Staff, United States Environmental Protection Agency, 1200 Pennsylvania Avenue (1105A), Washington, DC 20460, (202) 564-7333, Email: hq.foia@epa.gov, web page address: http://www.epa.gov/foia/.

Ohba, M. and D.H. Lee. 2003. *Bacillus thuringiensis* associated with faeces of the Kerama-jika, *Cervus nippon keramae*, a wild deer indigenous to the Ryukyus, Japan. J. Basic Microbiol. 43:158-162.

Ortega, F., C. Novillo, and P. Castanera. 1996. Characterization and distribution of digestive proteases of the stalk corn borer, *Sesamia nonagrioides* Lef. (Lepidoptera: Noctuidae). Arch. Insect Biochem. Physiol. 32:163-180.

Palm, C.J., D.L. Schaller, K.K. Donegan, and R.J. Seidler. 1996. Persistence in soil of transgenic plant produced *Bacillus thuringiensis* var. *kurstaki* δ-endotoxin. Can. J. Microbiol. 43:1258-1262.

Peacock, J.W., D.F. Schweitzer, J.L.Carter, and N.R. Dubois. 1998. Laboratory Assessment of the Effects of *Bacillus thuringiensis* on Native Lepidoptera. Environ. Entomol. 27:450-457.

Perlak, F.J., R.L. Fuchs, D.A.Dean, S.L. McPherson, and D.A. Fishhoff. 1991. Modification of the coding sequences enhances plant expression of insect control protein genes. Proc. Natl. Acad. Sci. (USA) 88:3324-3328.

Petras, S.F. and L.E. Casida, Jr. 1985. Survival of *Bacillus thuringiensis* spores in soil. App. Environ. Microbiol. 50:1496-1501.

Peyronnet, O., V. Vachon, R. Brousseau, D. Baines, J.L. Schwartz, and R. Laprade. 1997. Effect of *Bacillus thuringiensis* toxins on the membrane potential of lepidopteran insect midgut cells. App. Environ. Microbiol. 63:1679-1684.

Pierce, C.M.F., L.F. Solter, and R.A. Weinzierl. 2001. Interactions between *Nosema pyraustra* (Microsporidia: Nosematidae) and *Bacillus thuringiensis* subsp. *kurstaki* in the European corn borer (Lepidoptera: Pyralidae). J. Econ. Entomol. 94:1361–1368.

Pilcher, C.D., J.J. Obrycki, M.E. Rice, and L.C. Lewis. 1997. Preimaginal development, survival, and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn. Environ. Entomol. 26:446–454.

Pilcher, C. D., M.E. Rice, and J. J. Obrycki. 2005. Impact of Transgenic Bacillus thuringiensis Corn and Crop Phenology on Five Nontarget Arthropods. Environ. Entomol. 34:1302-1316.

Pittendrigh, B., P. Gaffney, J. Huesing, L. Onstad, and L. Murdock. 2004. Active refuges can inhibit the evolution of resistance in insects towards transgenic insect-resistant plants. J. Theor. Biol. 231:461-474.

Plant Genetic Systems (America) Inc. 1998a. USEPA Cry9C registration submission, MRID# 43843 (The MRID numbers provided represent a package of documents submitted to support a registration submission. See Tables and References in Annex for individual reports).*

Plant Genetic Systems (America) Inc. 1998b. USEPA Cry9C registration submission, MRID# 441617.*

Plant Genetic Systems (America) Inc. 1998c. USEPA Cry9C registration submission, MRID# 442581.*

Pleasants, J.M., R.L. Hellmich, G.P. Dively, M.K. Sears, D.E. Stanley-Horn, H.R. Mattila, J.E. Foster, P.Clark, and G.D. Jones. 2001. Corn pollen deposition on milkweeds in and near cornfields. Proc. Natl. Acad. Sci. (USA) 98:11919-11924.

Poncet, S., C. Bernard, E. Dervyn, J. Cayley, A. Klier, and G. Rapoport. 1997. Improvement of *Bacillus sphaericus* toxicity against dipteran larvae by integration, via homologous recombination, of the CryIIA toxin gene from *Bacillus thuringiensis* subsp. *israelensis*. Appl. Environ. Microbiol. 63:4413-4420.

Pons, X., B. Lumbierres, C. Lopez, and R. Albajes. 2005. Abundance of non-target pests in transgenic Btmaize: A farm scale study. Eur. J. Entomol. 102:73-79.

* Health and safety studies submitted to the US Environmental Protection Agency in support of pesticidal registration can be released to the general public (with a few restrictions) after the product is registered. These, as identified by a Master Record Identification (MRID) number, can be obtained through the Freedom of Information Office at: HQ FOIA Operations Staff, United States Environmental Protection Agency, 1200 Pennsylvania Avenue (1105A), Washington, DC 20460, (202) 564-7333, Email: hq.foia@epa.gov, web page address: http://www.epa.gov/foia/.

Ponsard, S., A.P. Gutierrez, and N.J. Mills. 2002. Effect of Bt-toxin (Cry1Ac) in transgenic cotton on the adult longevity of four heteropteran predators. Environ. Entom. 31:1197-1205.

Potenza, C., B.L. Aleman, and C. Sengupta-Gopalan. 2004. Inited review: Targeting transgene expression in research, agricultural and environmental applications: Promoters used in plant transformation. *In Vitro* Cell. Dev. Biol. Plant 40:1-22.

Poza, M. de la, X. Pons, G.P. Farinósa, C. López, F. Ortego, M. Eizaguirre, P. Castañera, and R. Albajes. 2005. Impact of farm-scale Bt maize on abundance of predatory arthropods in Spain. Crop Protect. 24:677-684.

Prasifka, J.R., R.L. Hellmich, G.P. Dively, and L.C. Lewis. 2005. Assessing the effects of eest management on nontarget arthropods: The influence of plot size and isolation. Environ. Entomol. 34:1181-1192.

Pratt, G.E., L.A. Royce, and B.A. Croft. 1993. Measurement of toxicity of soils following incorporation of plant residues engineered with *Bacillus thuringiensis* v. *kurstaki* endotoxin, using a *Heliothis virescens* growth bioassay. *In:* Proceedings of the Fifth Investigators Meeting for EPA's Environmental Release of Biotechnology Research Program, Duluth, MN.

Prieto-Samsonov, D.L., R.I. Vazquez-Padron, C.Ayra-Pardo, J. Gonzalez-Cabrera, and G.A. de la Riva. 1997. *Bacillus thuringiensis*: From biodiversity to biotechnology. J. Ind. Microbiol. Biotechnol. 19:202-219.

Promdonkoy, B. and D.J. Ellar. 2003. Investigation of the pore-forming mechanism of a cytolytic delta-endotoxin from *Bacillus thuringiensis*. Biochem. J. 374:255-259.

Prütz, G., A. Brink, and K. Dettner. 2004. Transgenic insect-resistant corn affects the fourth trophic level: effects of Bacillus thuringiensis-corn on the facultative hyperparasitoid *Tetrastichus howardi*. Naturwissenschaften 91:451-454.

Prütz, G. and K. Dettner. 2004. Effect of Bt corn leaf suspension on food consumption by *Chilo partellus* and life history parameters of its parasitoid *Cotesia flavipes* under laboratory conditions. Entomol. Exp. Appl. 111:179-187.

Rajamohan, F., M.K. Lee, and D.H. Dean. 1998. *Bacillus thuringiensis* insecticidal proteins: molecular mode of action. Prog. Nucleic Acid Res. Mol. Biol. 60:1-27.

Raps, A., J. Kehr, P. Gugerli, W.J. Moar, F. Bigler, F., and A. Hilbeck. 2001. Immunological analysis of phloem sap of *Bacilluis thuringiensis* corn and of the non-target herbivore *Rhopalosiphum padi* (Homoptera: Aphididae) for the presence of Cry1Ab. Mol. Ecol. 10:525-533.

Ravoahangimalala, O. and J.F. Charles. 1995. *In vitro* binding of *Bacillus thuringiensis* var. *israelensis* individual toxins to midgut cells of *Anopheles gambiae* larvae (Diptera: Culicidae). FEBS Lett. 362:111-115.

Ravoahangimalala, O., J.F. Charles, and J. Schoeller-Raccaud. 1993. Immunological localization of *Bacillus thuringiensis* serovar *israelensis* toxins in midgut cells of intoxicated *Anopheles gambiae* larvae Diptera:culicidae. Res. Microbiol. 144:271-278.

Raynor, G.S., J.V. Hayes, and E.C. Ogden. 1972. Dispersion and deposition of corn pollen from experimental sources. Agron. J. 64:420-426.

Reardon, B.J., R.L. Hellmich, D.V. Sumerford, and L.C. Lewis. 2004. Growth, development and survival of *Nosema pyraustra*-infected European corn borers (Lepidoptera: Crambidae) reared on meridic diet and Cry1Ab. J. Econ. Entomol. 97:1198–1201.

Reed, G.L., A.S. Jensen, J. Riebe, G. Head, and J.J. Duan. 2001. Transgenic potato and conventional insecticides for Colorado potato beetle management: comparative efficacy and non-target impacts. Entomol. Exp. Appl. 100:89-100.

Riddick, E.W. and P. Barbosa. 1998. Impact of Cry3A intoxicated *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) and pollen on consumption, development, and fecundity of *Coleomegilla maculata* (Coleoptera: Coccinellidae). Ann. Entomol. Soc. Am. 91:303-307.

Riddick, E.W. and P. Barbosa. 2000. Cry3A-intoxicated *Leptinotarsa decemlineata* (Say) are palatable prey for *Lebia grandis* Hentz. J. Entomol. Sci. 35:342-346.

Riddick, E.W., G. Dively, and P. Barbosa. 2000. Season-long abundance of generalist predators in transgenic *versus* non-transgenic potato fields. J. Entomol. Sci. 35:349-359.

Romeis, J., D. Bartsch, F. Bigler, M. Candolfi, M. Gielkens, S. Hartley, R. Hellmich, J. Huesing, P. Jepson, R. Layton, H. Quemada, A. Raybould, R. Rose, J. Schiemann, M. Sears, A. Shelton, J. Sweet, Z. Vaituzis, and J. Wolt. 2006a. Moving through the tiered and methodological framework for non-target arthropod risk assessment of transgenic insecticidal crops. Proceedings of the 9th International Symposium on the Biosafety of Genetically Modified Organisms, September 24-29, Jeju Island, Korea. p 62-67.

Romeis, J., A. Dutton, and F. Bigler. 2004. Bacillus thuringiensis toxin (Cry1Ab) has no direct effect on larvae of the green lacewing Chrysoperla carnea (Stephens) (Neuroptera: Chrysopidae). J. Insect Physiol. 50:175-183.

Romeis, J., M. Meissle, and F. Bigler. 2006b. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. Nat. Biotechnol. 24:63-71.

Rose, R. I. 2006. Tier-based testing for effects of proteinaceous insecticidal plant-incorporated protectants on non-target arthropods in the context of regularoty risk assessments. GMOs in Integrated Plant Production: OBC/wprs Bull. 29:143-150.

Rovenská, G.Z., R. Zemek, J.E.U. Schmidt, and A. Hilbeck. 2005. Altered host plant preference of *Tetranychus urticae* and prey preference of its predator *Phytoseiulus persimilis* (Acari: Tetranychidae, Phytoseidae) on transgenic Cry3Bb-eggplants. Biol. Control 33:293-300.

Ruhfel, R., N.J. Robillard, and C.B. Thorne. 1984. Interspecies transduction of plasmids among *Bacillus anthracis, B. cereus,* and *B. thuringiensis.* J. Bacteriol. 157:708-711.

Rukmini, V., C.Y. Reddy, and G. Venkateswerlu. 1999. *Bacillus thuringiensis* crystal δ-endotoxin: Role of proteases in the conversion of protoxin to toxin. Biochimie 82:109-116.

Salamitou, S., F. Ramisse, M. Brehelin, D. Bourguet, N. Gilois, M. Gominet, E. Hernandez, and D. Lereclus. 2000. The plcR regulon is involved in the opportunistic properties of *Bacillus thuringiensis* and *Bacillus cereus* in mice and insects. Microbiology 146:2825-2832.

Sanvido, O., M. Stark, J. Romeis, and F. Bigler. 2006. Ecological impacts of genetically modified crops: Experiences from ten years of experimental field research and commercial cultivation. Swiss Confederation, Federal Department of Economic Affairs DEA, Agroscope Reckenholz-Tanikon Research Station (ART), Zurich. 86 p.

Saxena. D., S. Flores, and G. Stotzky. 1999. Insecticidal toxin in root exudates from Bt corn. Nature 402:480.

Saxena. D., S. Flores, and G. Stotzky. 2002a. Bt toxin released in root exudates from 12 transgenic corn hybrids representing three transformation events. Soil Biol. Biochem. 34:133-137.

Saxena. D., S. Flores, and G. Stotzky. 2002b. Vertical movement in soil of insecticidal Cry1Ab protein from *Bacillus thuringiensis*. Soil Biol. Biochem. 34:111-120.

Saxena. D. and G. Stotzky. 2000. Insecticidal toxin from *Bacillus thuringiensis* is released from roots of transgenic Bt corn *in vitro* and *in situ*. FEMS Microbiol. Ecol. 33:35-39.

Saxena. D. and G. Stotzky. 2001. *Bacillus thuringiensis* (Bt) toxin released from roots exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil. Soil Biol. Biochem. 33:1225-1230.

Sears, M.K., R.L. Hellmich, D.E. Stanley-Horn, K.S. Oberhauser, J.M. Pleasants, H.R. Mattila, B.D. Siegfried, and G.P. Dively. 2001. Impact of Bt corn pollen on monarch butterfly populations: A risk assessment. Proc. Natl. Acad. Sci. (USA) 98:11937-11942.

Shivakumar, A.G., G.J. Gundling, T.A. Benson, D. Casuto, M.F. Miller, and B.B. Spear. 1986. Vegetative expression of the delta-endotoxin genes of *Bacillus thuringiensis* subsp. *kurstaki* in *Bacillus subtilis*. J. Bacteriol. 166:194-204.

Schmitz, G., P. Pretscher, and D. Bartsch. 2003. Selection of relevant non-target herbivores for monitoring the environmental effects of Bt maize pollen. Environ. Biosafety Res. 2:117-132.

Schnepf, E., N. Crickmore, J. Van Rie, D. Lareclus, J. Baum, J. Feitelson, D.R. Zeigler, and D.H. Dean. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol. Mol. Biol. Rev. 62:775-806.

Schwartz, J.L., L. Potvin, X.J. Chen, R. Brousseau, R. Laprade, and D.H. Dean. 1997. Single-site mutations in the conserved alternating-arginine region affect ionic channels formed by CryIAa, a *Bacillus thuringiensis* toxin. Appl. Environ. Microbiol. 63:3978-3984.

Selinger, L.B., G.G. Khachatourians, J.R. Byers, and M.G. Hynes. 1998. Expression of a *Bacillus thuringiensis* δ-endotoxin gene by *Bacillus pumilis.* Can. J. Microbiol. 44:259-269.

Shirai, Y. and M. Takahashi. 2005. Effects of transgenic corn pollen on a non-target lycaenid butterfly, *Pseudozizeeria maha*. Appl. Entomol. Zool. 40:151-159.

Siegel, J.P. 2001. The mammalian safety of *Bacillus thruingiensis*-based insecticides. J. Invertebr. Pathol. 77:13-21.

Siegfried, B.D., A.C. Zoerb, and T. Spencer. 2001. Development of European corn borer larvae on Event 176 Bt corn: influence on survival and fitness. Entomol. Exp. Appl. 100:15-20.

Simpson, R.M. and R.D. Newcomb. 2000. Binding of *Bacillus thuringiensis* delta-endotoxins Cry1Ac and Cry1Ba to a 120-kDa aminopeptidase-N of *Epiphyas postvittana* purified from both brush border membrane vesicles and baculovirus-infected Sf9 cells. Insect Biochem. Mol. Biol. 11:1069-1078.

Sims, S.R. 1995. *Bacillus thuringiensis* var *kurstaki* [CryIA(c)] protein expressed in transgenic cotton: Effects on beneficial and other non-target insects. Southwest. Entomol. 20:493-500.

Sisterson, M.S., R.W. Biggs, C. Olson, Y. Carrière, T.J Dennehy, and B.E. Tabashnik. 2004. Arthropod abundance and diversity in Bt and non-Bt cotton fields. Environ. Entomol. 33:921-929.

Sjoblad, R.D., J.T. McClintock, and R. Engler. 1992. Toxicological considerations for protein components of biological pesticide products. Regul. Toxicol. Pharmacol. 15:3-9.

Smith, R.A. and G.A. Couche. 1991. The phylloplane as a source of *Bacillus thuringiensis* variants. App. Environ. Microbiol. 57:311-315.

Snow, A.A., D. Pilson, L.H. Riesenberg, M.J. Paulsen, N. Pleskac, M.R. Reagon, D.E. Wolf, and S.M. Selbo. 2003. A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers. Ecol. Appl. 13:279-286.

Soberon, M., R.V. Perez, M.E. Nunez-Valdez, A. Lorence, I. Gomez, J. Sanchez, and A. Bravo. 2000. Evidence for intermolecular interaction as a necessary step for pore-formation activity and toxicity of *Bacillus thuringiensis* Cry1Ab toxin. FEMS Microb. Lett. 191:221-225.

Stanley-Horn, D.E., G.P. Dively, R.L. Hellmich, H.R. Mattila, M.K. Sears, R. Rose, L.C.H. Jesse, J.E. Losey, J.J. Obrycki, and L. Lewis. 2001. Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. Proc. Natl. Acad. Sci. (USA) 98:11931-11936.

Stone, T.B., S.R. Sims, and P.G. Marrone. 1989. Selection of tobacco budworm for resistance to a genetically engineered *Pseudomonas fluorescens* containing the δ-endotoxin of *Bacillus thuringiensis* subsp. *kurstaki*. J. Invertebr. Pathol. 53:228-234.

Stotzky, G. 2000. Persistence and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis* and of bacterial DNA bound on clays and humic acids. J. Environ. Qual. 29:691-705.

Stotzky, G. 2002. Release, persistence, and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis*. *In*: Letournear, D.K. and B.E. Burrows (Eds.), Genetically Engineered Organisms: Assessing Environmental and Human Health Effects. CRC Press, Boca Raton, Fla. p 187-222.

Sutton, S.A., A.H Assa'ad, C. Steinmetz, and M.E. Rothenberg. 2003. A negative, double-blind, placebo-controlled challenge to genetically modified corn. J. Allergy Clin. Immunol. 112:1011-1012.

Swiecicka, I. and P. De Vos. 2003. Properties of *Bacillus thuringiensis* isolated from bank voles. J. Appl. Microbiol. 94:60-64.

Swiecicka, I., K. Fiedoruk, and G. Bednarz. 2002. The occurrence and properties of *Bacillus thuringiensis* isolated from free-living animals. Lett. Appl. Microbiol. 34:194.

Szekacs, A., J. Juracsek, L.A. Polgar, and B. Darvas. 2005. Levels of expressed Cry1Ab toxin in genetically modified corn DK-440-BTY (YIELDGARD) and stubble. FEBS Journal 272 (s1), L3-005. (http://www.blackwellpublishing.com/febsabstracts2005/abstract.asp?id=41771).

Tabashnik, B.E. 1992 Evaluation of synergism among *Bacillus thuringiensis* toxins. Appl. Environ. Microbiol. 58:3343-3346.

Tabashnik, B.E., Y. Carrière, T.J. Dennehy, S. Morin, M.S. Sisterson, R.T. Roush, A.M. Shelton, and J.Z. Zhao. 2003. Insect resistance to transgenic Bacillus thuringiensis crops: lessons from the laboratory and field. J. Econ. Entomol. 96:1031–1038.

Tabashnik, B.E., N.L. Cushing, N. Finson, and M.W. Johnson. 1990. Field development of resistance to *Bacillus thuringiensis* in the diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 83:1671-1676.

Tapp, H. and G. Stotzky. 1995a. Dot blot enzyme-lynked immunosorbent assay for monitoring the fate of insecticidal toxins from *Bacillus thuringiensis* in soil. Appl. Environ. Microbiol. 61:602-609.

Tapp, H. and G. Stotzky. 1995b. Insecticidal activity of the toxins from *Bacillus thuringiensis* subspecies *kurstaki* and *tenebrionis* adsorbed and bound on pure and soil clays. Appl. Environ. Microbiol. 61:1786-1790.

Tapp, H. and G. Stotzky. 1997. Monitoring the insecticidal toxins from *Bacillus thuringiensis* in soil with flow cytometry. Can. J. Microbiol. 43:1074-1078.

Ticknor, L.O., A. Kolsto, K.K. Hill, P. Keim, M.T. Laker, M. Tonks, and P.J. Jackson. 2001. Fluorescent AFLP analysis of norwegian *Bacillus cereus and Bacillus thuringiensis* soil isolates. Appl. Environ. Microbiol. 67: 4863-4873.

Torres, J.B. and J.R. Ruberson. 2005. Canopy- and ground-dwelling predatory arthropods in commercial Bt and non-Bt cotton fields: Patterns and mechanisms. Environ. Entomol. 34:1242-1256.

Tran, L.B., V. Vachon, J-L. Schwartz, and R. Laprade. 2001. Differential effects of pH on the pore-forming properties of *Bacillus thuringiensis* insecticidal crystal toxins. Appl. Environ. Microbiol. 67:4488-4494.

Treu, R. and J. Emberlin. 2000. Pollen dispersal in the crops maize (*Zea mays*), oil seed rape (*Brassica napus* spp. *oleifera*), potatoes (*Solanum tuberosum*), sugar beet (*Beta vulgaris* ssp. *vulgaris*) and wheat (*Triticum aestivum*). Soil Association, Bristol, UK. 2 p. Available from: http://www.soilassociation.org/web/sa/saweb.nsf/librarytitles/22DD2.HTMl/$file/Pollen%20dispersal,%20npru,%202000.pdf

United States Pharmacopeia. 1995. United States Pharmacopeial Convention, Inc. Rockville, MD. 23:2053

USEPA (United States Environmental Protection Agency). 1998. Reregistration Eligibility Decision (RED) *Bacillus thuringiensis*. EPA-738-R-98-004. 170 p. Available from: www.epa.gov/oppsrrd1/REDs/0247.pdf

USEPA. 2000. Bt Plant-Pesticides Biopesticides Registration Document: Section C. Environmental Assessment. United States Environment Protection Agency, Washington, DC.

USEPA. 2001. *Bacillus thruingiensis* (*Bt*) Plant-Incorporated Protectants October 15, 2001, Biopesticides Registration Action Document. U.S. Environmental Protection Agency, Washington, DC. 30 p. Available from: http://www.epa.gov/pesticides/biopesticides/pips/bt_brad2/1-overview.pdf

USEPA. 2001. Biopesticides Registration Action Document – *Bacillus thruingiensis* Plant-Incorporated Protectants [October 15, 2001]. Available from: http://www.epa.gov/pesticides/biopesticides/pips/bt_brad.html

USEPA. 2003a. Event MON863 Bt Cry3Bb1 Corn Biopesticide Registration Action Document. United States Environment Protection Agency, Washington, DC. Available from: http://www.epa.gov/oppbppd1/biopesticides/ingredients/tech_docs/brad_006484.htm.

USEPA. 2003b. Microbial Pesticide Test Guidelines – Overview. Available from: http://www.epa.gov/pesticides/biopesticides/regtools/guidelines/oppts_885_0001.htm

USEPA. 2005. Biopesticides Active Ingredient Fact Sheets – *Bacillus thuringiensis* Cry34Ab1 and Cry35Ab1 proteins and the genetic material necessary for their production (plasmid insert PHP 17662) in Event DAS-

59122-7 corn. Avaliable from: http://www.epa.gov/oppbppd1/biopesticides/ingredients/factsheets/factsheet_006490.htm

Vacek, M., A. Reynaerts, H. Hofte, S. Jansens, M. De Beukeleer, C. Dean, M. Zabeau, M. Montagu, and J. Leemans. 1987. Transgenic plants protected from insect attack. Nature 328:33-37.

Vacher, C., A.E. Weis, D. Hermann, T. Kossler, C. Young, and M.E. Hochberg. 2004. Impact of ecological factors on the initial invasion of *Bt* transgenes into wild populations of birdseed rape (*Brassica rapa*). Theor. Appl. Gene. 109:806-814.

Vadlamudi, R.K., E. Weber, I. Ji, T.H. Ji, and L.A. Bulla, Jr. 1995. Cloning and expression of a receptor for an insecticidal toxin of *Bacillus thruingiensis*. J. Biol. Chem. 270:5490-5494.

Van Rie, J., S. Jansens, H. Hofte, D. Degheele, and H. Van Mellaert. 1989. Specificity of *Bacillus thuringiensis* delta-endotoxins. Eur. J. Biochem. 186:239-247.

Van Rie, J., S. Jansens, H. Hofte, D. Degheele, and H. Van Mellaert. 1990a. Receptors on the brush border membrane of the insect midgut as determinants of the specificity of *Bacillus thuringiensis* delta-endotoxins. Appl. Environ. Microbiol. 56:1378-1385.

Van Rie, J., W.H. McGaughey, D.E. Johnson, B.D. Barnett, and H. Van Mellaert. 1990b. Mechanism of insect resistance to the microbial insecticide *Bacillus thuringiensis*. Science 247:72-74.

Vercesi, M., P. Krogh, and M. Holmstrup. 2006. Can *Bacillus thuringiensis* (Bt) corn residues and Bt-corn plants affect life-history traits in the earthworm *Aporrectodea caliginosa*? Appl. Soil Ecol. 32:180-187.

Vettori, C., D. Paffetti, D. Saxena, G. Stotzky, and R. Giannini. 2003. Persistence of toxins and cells of *Bacillus thuringiensis* subsp. *kurstaki* introduced in sprays to Sardinia soils. Soil Biol. Biochem. 35:1635-1642.

Visser, S., J.A. Addison, and S.B. Holmes. 1994. Effects of DiPel 176, a *Bacillus thuringiensis* subsp. *kurstaki (B.t.k.)* formulation, on the soil microflora and the fate of *B.t.k.* in an acid forest soil: a laboratory study. Can. J. For. Res. 24:462-471.

Vitale, A. and J. Denecke. 1999. The endoplasmic reticulum - gateway to the secretory pathway. Plant Cell 11:615-628.

Vojtech, E., M. Meissle, and G.M. Poppy. 2005. Effects of Bt maize on the herbivore *Spodoptera littoralis* (Lepidoptera: Noctuidae) and the parasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae). Transgenic Res. 14:133-144.

Von Tersch, M.A., H.L. Robbins, C.S. Jany, and T.B. Johnson. 1991. Insecticidal toxins from *Bacillus thuringiensis* subsp. *kenyae*: Gene cloning and characterization and comparison with *B. thuringiensis* subsp. *kurstaki* CryIA(c) toxins. Appl. Environ. Microbiol. 57:349-358.

Von Tersch, M.A., S.L. Slatin, C.A. Kulesza, and L.H. English. 1994. Membrane-permeabilizing activities of *Bacillus thuringiensis* coleopteran-active toxin CryIIIB2 and CryIIIB2 domain I peptide. Appl. Environ. Microbiol. 60:3711-3717.

Walters, F.S., S.L. Slatin, C.A. Kulesze, and L.H. English. 1993. Ion channel activity of n-terminal fragments from the CryIA(c) delta-endotoxin. Biochem. Biophys. Res. Comm. 196:921-926.

Wagner, D.L., J.W. Peacock, J.L. Carter, and S.E. Talley. 1996. Field assessment of *Bacillus thuringiensis* on non-target Lepidoptera. Environ. Entomol. 25:1444-1454.

Wagner, W., F. Mohrlen, W. Schnetter. 2002. Characterization of the proteolytic enzymes in the midgut of the European Cockchafer, *Melolontha melolontha* (Coleoptera: Scarabaeidae). Insect Biochem. Mol. Biol. 32:803-814.

Wan, F.H., W.X. Liu, and J.Y. Guo. 2002. Comparison analyses of the functional groups of natural enemy in transgenic Bt cotton field and non-transgenic cotton fields with IPM, and chemical control. Acta Ecol. Sin. 22:935-942.

Wandeler, H., J. Bahylova, and W. Nentwig. 2002. Consumption of two Bt and six non-Bt corn varieties by the woodlouse *Porcellio scaber*. Basic Appl. Ecol. 3:357-365.

Wang, C.Y. and J.Y. Xia. 1997. Differences of population dynamics of boll-worms and of population dynamics of major natural enemies between Bt transgenic cotton and conventional cotton. China Cotton 24:13-15.

Wang S.W. and W.J. McCarthy. 1997. Cytolytic activity of *Bacillus thuringiensis* CryIC and CryIAc toxins to *Spodoptera* sp. Midgut epithelial cells *in vitro*. *In Vitro* Cell Dev. Biol. Anim. 33:315-323.

Whitehouse, M.E.A., L.J. Wilson, and G.P. Fitt. 2005. A Comparison of Arthropod Communities in Transgenic Bt and Conventional Cotton in Australia. Environ. Entomol. 34:1224-1241.

Widner, W.R. and H.R. Whiteley. 1989. Two highly related insecticidal crystal proteins of *Bacillus thuringiensis* subsp. *kurstaki* possess different host range specificities. J Bacteriol. 171:965-74.

Widner, W.R. and H.R. Whiteley. 1990. Location of the dipteran specificity region in a lepidopteran-dipteran crystal protein from *Bacillus thuringiensis*. J Bacteriol. 172:2826-32.

Williams, S., L. Friedrich, S. Dincher, N. Carozzi, H. Kessmann, E. Ward, and J. Ryals. 1992. Chemical regulation of *Bacillus thuringiensis* δ-endotoxin expression in transgenic plants. Biotechnology 10:540-543.

Wold, S.J., E.C. Burkness, W.D Hutchison, and R.C. Venette. 2001. In-field monitoring of beneficial insect populations in transgenic corn expressing a *Bacillus thuringiensis* toxin. J. Entomol. Sci. 36:117-187.

Wolt, J.D., R.K.D. Peterson, P. Bystrak, and T. Meade. 2003. A screening level approach for non-target risk assessment: transgenic Bt corn pollen and the monarch butterfly (Lepidoptera: Danaidae). Environ. Entomol. 32:237-246.

Wolt, J.D., C.A. Conlan, and K. Majima. 2005. An ecological risk assessment of Cry1F maize pollen impact to pale grass blue butterfly. Environ. Biosafety Res. 4:243-251.

Wraight, C.L., A.R. Zangerl, M.J. Carroll, and M.R. Berenbaum. 2000. Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions. Proc. Natl. Acad. Sci. (USA) 97:7700–7703.

Wu, K. and Y. Guo. 2003. Influences of *Bacillus thuringiensis* Berliner cotton planting on population dynamics of the cotton aphid, *Aphis gossypii* Glover, in Northern China. Environ. Entomol. 32:312-318.

Wu, K., W. Li, H. Feng, and Y. Guo. 2002. Seasonal abundance of the mirids, *Lygus lucorum* and *Adelphocoris* spp. (Hemiptera: Miridae) on Bt cotton in northern China. Crop Protect. 21:997-1002.

Yaoi, K., K. Nakanishi, T. Kadotani, M. Imamura, N. Koizumi, H. Iwahana, and R. Sato. 1999. cDNA cloning and expression of *Bacillus thuringiensis* Cry1Aa toxin binding 120 kDa aminopeptidase N from *Bombyx mori*. Biochim. Biophys. Acta 1444:131-137.

Yu, C., M.A. Mullins, G.W. Warren, M.G. Koziel, and J.J. Estruch. 1997. The *Bacillus thuringiensis* vegetative insecticidal protein Vip3A lyses midgut epithelium cells of susceptible insects. Appl. Environ. Microbiol. 63:532-536.

Yu, L., R.E. Berry, and B.A. Croft. 1997. Effects of *Bacillus thuringiensis* toxins in transgenic cotton and potato on *Folsomia candida* (*Collembola: Isotomidae*) *Oppia nitens* (*Acari: Orbatidae*). J. Econ. Entom. 90:113-118.

Zangerl, A.R., D. McKenna, C.L. Wraight, M. Carroll, P. Ficarello, R. Warner, and M.R. Berenbaum. 2001. Effects of exposure to event 176 *Bacillus thuringiensis* corn pollen on monarch and black swallowtail caterpillars under field conditions. Proc. Natl. Acad. Sci. (USA) 98:11908-11912.

Zhang, J., T.C. Hodgman, L. Krieger, W. Schnetter, and H.U. Schairer. 1997. Cloning and analysis of the first *cry* gene from *Bacillus popilliae*. J. Bacteriol. 179:4336-4341.

Zhu, Y.C., K.J. Kramer, B.Oppert, and A.K. Dowdy. 2000. cDNAs of aminopeptidase-like protein genes from *Plodia interpunctella* strains with different susceptibilities to *Bacillus thuringiensis* toxins. Insect Biochem. Mol. Biol. 3:215-24.

Zscheischler, J., M.C. Estler, W. Staudacher, F. Groß, G. Burgstaller, H. Streyl, and T. Rechenmann. 1990. Handbuch Mais: umweltgerechter Anbau; wirtschaftliche Bewertung. München, DLG-Verlag.

Zwahlen, C. and D. Andow. 2005. Field evidence for the exposure of ground beetles to Cry1Ab from transgenic corn. Environ. Biosafety Res. 4:113-117.

Zwahlen, C., A. Hilbeck, R. Howald, and W. Nentwig. 2003a. Effects of transgenic Bt corn litter on the earthworm *Lumbricus terrestris*. Mol. Ecol. 12:1077-1086.

Zwahlen, C., A. Hilbeck, P. Guggerli, and W. Nentwig. 2003b. Degradation of the Cry1Ab protein within transgenic *Bacillus thuringiensis* corn tissue in the field. Mol. Ecol. 12:765-775.

Zwahlen, C., W. Nentwig, F. Bigler, and A. Hilbeck. 2000. Tritrophic interactions of transgenic *Bacillus thuringiensis* corn, *Anaphothrips obscurus* (Thysanoptera: Thripidae), and the predator *Orius majusculus* (Heteroptera: Anthocoridae). Environ. Entomol. 29:846-850.

Additional references

During final review of the document, additional references were suggested by reviewing countries. Since all countries would not have the chance to review their content, these references are added as a supplemental list to serve as an additional resource for the document.

Section 1 – General introduction

Brubaker, C.L., A.H.D. Brown, J.McD. Stewart, M.J. Kilby, and J.P. Grace. 1999. Production of fertile hybrid germplasm with diploid Australian *Gossypium* species for cotton improvement. Euphytica. 108:199-214.

Eastick, R. and M. Hearnden. 2006. Potential for weediness of Bt cotton in northern Australia. Weed Science 54:1142-1151.

Greenplate, J.T., J.W. Mullins, S.R. Penn, A. Dahm, B.J. Reich, J.A. Osborn, P.R. Rahn, L. Ruschke, and Z.W. Shappley. 2003. Partial characterization of cotton plants expressing two toxin proteins from *Bacillus thuringiensis*: relative toxin contribution, toxin interaction, and resistance management. J. Appl. Entomol. 127:340-347.

Rogers, D., R. Reid, J. Rogers, and S. Addison. 2007. Prediction of the naturalisation potential and weediness risk of transgenic cotton in Australia. Agric. Ecosyst. Environ. 119:177-189.

Section 1.2. Bacillus thuringiensis toxins

Baum J., C. Chu, M. Rupar, G.R. Brown, W.P. Donovan, J. Huesing, O. Ilagan, T. Malvar, M. Pleau, M. Walters, and T. Vaughn. 2004. A new binary toxin from *Bacillus thuringiensis* active against the western corn rootworm, *Diabrotica virgifera virgifera*. Appl. Environ. Microbiol. 70:4889-4898.

Ellis, R.T., B.A. Stockhoff, L. Stamp, H.E. Schnepf, G.E. Schwab, M. Kunth, J. Russell, G.A. Cardineau, and K.E. Narva. 2002. Novel *Bacillus thuringiensis* binary insecticidal crystal proteins active on western corn rootwoom, *Diabrotica virgifera virgifera* LeConte. App. Environ. Microbiol. 68:1137-1145.

Herman, R.A., P.N. Scherer, D.L. Young, C.A. Mihaliak, T. Meade, A.T. Woodsworth, B.A. Stockhoff, and K.E. Narva. 2002. Binary insecticidal crystal protein from *Bacillus thuringiensis*, strain PS149B1: effects of individual protein components and mixtures in laboratory bioassays. J. Econ. Entomol. 95:635-639.

Mollenbeck, D.J., M.L. Peters, J.W. Bing, J.R. Rouse, L.S. Higgins, L. Sims, T. Nevshemal, L. Marshall, R.T. Ellis, P.G. Bystrak, B.A Lang, J.L. Stewart, K. Koubal, V. Sondag, V. Gustafson, K. Nour, D. Xu, J. Swenson, J. Zhang, T. Czapia, G. Schwab, S. Jayne, B.A. Stockhoff, K. Narva, H.E. Schnepf, S.J. Stelman, C. Poutre, M. Koziel, and N. Duck. 2001. Insecticidal proteins from *Bacillus thuringiensis* protect corn from corn rootworms. Nat. Biotech. 19:668-672.

Schnepf, H.E., S. Lee, J. Dojillo, P. Burmeister, K. Fencil, L. Morera, L. Nygaard, K.E. Narva, and J.D. Wolt. 2005. Characterization of Cry34/Cry35 binary insecticidal proteins from diverse *Bacillus thuringiensis* strain collections. App. Environ. Microbiol. 71:1765-1774.

Section 1.3. Susceptible insects

Crickmore, N., V. Wheeler, and D.J. Ellar. 1994. Use of an operon fusion to induce expression and crystallization of a *Bacillus thuringiensis* delta-endotoxin encoded by a cryptic gene. Mol. Gen. Genet. 242:365-368.

Donovan, W.P., C. Dankocsik, M.P. Gilbert, M.C. Gawron-Burke, R.G. Groat, and B.C. Carlton. 1988. Amino acid sequence and entomocidal activity of the P2 crystal protein, an insect toxin from *Bacillus thuringiensis* var. *kurstaki*. J. Biol. Chem. 263:561-567.

Section 3.3. Structure of toxins

Boonserm, P., P. Davis, D.J. Ellar, and J. Li. 2005. Crystal structure of the mosquito-larvicidal toxin Cry4Ba and its biological implications. J. Mol. Biol. 348:363-382.

Boonserm, P., M. Mo, C. Angsuthanasombat, and J. Lescar. 2006. Structure of the functional form of the mosquito larvicidal Cry4Aa toxin from *Bacillus thuringiensis* at a 2.8-angstrom resolution. J. Bacteriol. 188:3391-3401.

Derbyshire, D.J., D.J. Ellar, and J. Li. 2001. Crystallization of the *Bacillus thuringiensis* toxin Cry1Ac and its complex with the receptor ligand N-acetyl-D-galactosamine. Acta Cryst. Sect. D 57:1938-1944.

Li, J., D.J. Derbyshire, B. Promdonkoy, and D.J. Ellar. 2001. Structural implications for the transformation of the *Bacillus thuringiensis* δ-endotoxins from water-soluble to membrane-inserted forms. Biochem. Soc. Trans. 29**:** 571-577.

Pigott, C. and D.J. Ellar. 2007. Role of receptors in *Bacillus thuringiensis* crystal toxin activity. Microbiol. Mol. Biol. Rev. 71:255-281.

Section 4 – Mechanism of action

Bravo, A., I. Gomez, J. Conde, C. Munoz-Garay, J. Sanchez, R. Miranda, M. Zhuang, S.S. Gill, and M. Soberon. 2004. Oligomerization triggers binding of a *Bacillus thuringiensis* Cry1Ab pore-forming toxin to aminopeptidase N receptor leading to insertion into membrane microdomains. Biochim. Biophys. Acta 1667:38-46.

Jurat-Fuentes, J.L. and M. Adang. 2006. Cry toxin mode of action in susceptible and resistant *Heliothis virescens* larvae. J. Invertebr. Pathol. 92:166-171.

Knowles, B. and D.J. Ellar. 1987. Colloid-osmotic lysis is a general feature of the mechanism of action of *Bacillus thuringiensis* δ -endotoxins with different insect specificity. Biochim. Biophys. Acta 924:507-518.

Pigott, C. and D.J. Ellar. 2007. Role of receptors in *Bacillus thuringiensis* crystal toxin activity. Microbiol. Mol. Biol. Rev. 71:255-281.

Zhang, X., M. Candas, N. Griko, R. Taussig, and L. Bulla, Jr. 2006. A mechanism of cell death involving an adenylyl cyclase/PKA signaling pathway is induced by the Cry1Ab toxin of *Bacillus thuringiensis*. Proc. Natl. Acad. Sci. (USA) 103:9897-9902.

Section 4.2. Binding to receptors

Smedley, D.P. and D.J. Ellar. 1996. Mutagenesis of three surface-exposed loops of a Bacillus thuringiensis insecticidal toxin reveals residues important for toxicity, receptor recognition and possibly membrane insertion. Microbiol. 142: 1617-1624.

Maagd, R.A. de, M.S.G. Kwa, H. van der Klei, T. Yamamoto, B. Schipper, J.M. Vlak, W.J. Stiekema, and D. Bosch. 1996. Domain III substitution in *Bacillus thuringiensis* delta-endotoxin CryIA(b) results in superior toxicity for *Spodoptera exigua* and altered membrane protein recognition. Appl. Environ. Microbiol. 62:1537-1543.

Pigott, C. and D.J. Ellar. 2007. Role of receptors in *Bacillus thuringiensis* crystal toxin activity. Microbiol. Mol. Biol. Rev. 71:255-281.

Section 6.3. Non-target species

Cortet, J., M. Andersen, S. Caul, B. Griffiths, R. Joffre, B. Lacroix, C. Sausse, J.E. Thompson, and P. Krogh. 2006. Decomposition processes under Bt (*Bacillus thuringiensis*) maize: results of a multi-site experiment. Soil Biol. Biochem. 38:195-199.

Duan,J.J., C. Jiang, G.P. Head, M.A. Bhatti, D.P. Ward, S.L. Levine, T.E. Nickson, and M.A. Nemeth. 2006. Statistical power analysis of a 2-year field study and design of experiments to evaluate non-target effects of genetically modified *Bacillus thuringiensis* corn. Ecol. Entomol. 31:521-531.

Duan, J.J., J.E. Huesing, and D. Teixeira. In Press. Development of tier-I toxicity assays for *Orius insidiosus* (Heteroptera: Anthocoridae) for assessing the risk of plant-incorporated protectants to nontarget Heteropterans. Environ. Entomol. 36.

Garcia-Alonso, M., E. Jacobs, A. Raybould, T. Nickson, P. Sowig, H. Willekens, P. Van der Kouwe, R. Layton, F. Amijee, A. Fuentes, and F. Tencalla. 2007. A tiered system for assessing the risk of genetically modified plants to non-target organisms. Environ. Biosafety Res. 5:57-65.

Griffiths, B., S. Caul, J. Thompson, A. Birch, C. Scrimgeour, M. Andersen, J. Cortet, A. Messean, C. Sausse, B. Lacroix, P. Krogh. 2005. A comparison of soil microbial community structure, protozoa and nematodes in field plots of conventional and genetically modified maize expressing the *Bacillus thuringiensis* Cry1Ab toxin. Plant and Soil 275: 135-146.

Lang, A., E. Lauber, and B. Darvas. 2007. Early-field tests insufficient for GMO risk assessment. Nat. Biotech. 25: 35-36.

Marvier, M., C. McCreedy, J. Regetz, and P. Kareiva1. 2007. A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. Science 316:1475-1477.

Wolt, J., C. Conlan, and K. Majima. 2005. An ecological risk assessment of Cry1F maize pollen impact to pale grass blue butterfly. Environ. Biosafety Res. 4:243-251.

Section 6.4. Other ecological issues

Greenplate, J.T., J.W. Mullins, S.R. Penn, A. Dahm, B.J. Reich, J.A. Osborn, P.R. Rahn, L. Ruschke, and Z.W. Shappley. 2003. Partial characterization of cotton plants expressing two toxin proteins from *Bacillus thuringiensis*: relative toxin contribution, toxin interaction, and resistance management. J. Appl. Entomol. 127:340-347.

Annex I

Examples of company sponsored studies submitted in support of their product

The following tables reference these studies by their US identification numbers since the identification numbers as used by other countries are not available at this time. Studies judged as inadequate by USEPA reviewers are not included in these tables. Some of these studies in this document were submitted for products that have since been withdrawn or are in the process of being withdrawn from registration. However, these studies are still useful as general information on δ-endotoxins as a class. These studies are identified by a Master Record Identification (MRID) number which is used to locate them in the file system. They are available to the public. The best way to obtain the information (because of US legal restrictions) is through the Freedom of Information Office at: HQ FOIA Operations Staff, United States Environmental Protection Agency, 1200 Pennsylvania Avenue (1105A), Washington, DC 20460, (202) 564-7333, Email: hq.foia@epa.gov, web page address: http://www.epa.gov/foia/.

Table A. Studies submitted to and reviewed by USEPA in support of registration of Cry1Ab products

Assay	Toxin derived from Microbes (MT) or Plants (PT)	Results	USEPA - MRID Number
microbial (MT) and plant toxin (PT) equivalence (* + ELISA)	MT expressed in E.coli + PT	MT and PT are equivalent (6444)	433972-02
microbial and plant toxin equivalence (*)	MT from Dipel + PT	MT and PT are equivalent (6430)	435332-03
acute oral, mice		no effect>4000 mg/kg	434680-01
acute oral, mice	MT	no effect>3280 mg/kg	433236-08
acute oral, mice	PT	no effect>5050 mg/kg	434175-02
digestibility	MT and PT	degraded by pepsin	433236-06
digestibility + heat stability	MT	degraded by gastric fluid but not intestinal fluid - inactivated in processed maize and cottonseed meal	434392-01
acute oral, quail	PT in corn meal	no effect> 100,000 ppm maize grain	435332-05
acute oral, quail	PT	no effect>2000mg/kg	433236-09
adult honey bee	MT	no effect>20ppm	434392-03
honey bee larvae	MT	no effect>20ppm	434392-02
honey bee larvae	PT in pollen	no effect	434157-03
ladybird beetle	MT	no effect>20ppm	434680-05
ladybird beetle	PT in pollen	no effect	433396-02
green lacewing larvae	MT	no effect>20ppm	434680-03
parasitic wasp	MT	no effect>20ppm	434680-05
daphnia	MT	no effect>150mg/l	433236-10
collembola, 2 species	MT	no effect >200ppm	439416-01
collembola	PT	LD_{50} 240mg/kg/soil NOEL 125mg/kg/soil	434635-01
collembola, chronic	PT	no effect, including reproduction>50% of diet	442715-01
catfish	PT in corn meal	no effect at 100%of diet	438879-01
earthworm	MT	non-toxic	433396-01
earthworm	MT	non-toxic>200ppm	438879-02

* SDS-PAGE, Western blot, N-terminal amino acid sequencing, glycosylation, and bioactivity

Table B. Studies submitted to and reviewed by USEPA in support of registration of Cry1Ac products

Assay	Toxin derived from Microbes (MT) or Plants (PT)	Results	USEPA MRID Number
microbial and plant toxin equivalence (*)	MT expressed in E.coli + PT	MT and PT are equivalent (6445)	431452-02
acute oral, mice	MT	no effect>4200 mg/kg	431452-13
acute oral, mice	MT	no effect>5000 mg/kg	439995-01
digestibility		degraded by pepsin	439995-03
digestibility + heat stability	PT in diet	degraded by gastric fluid inactive in processed cottonseed meal	431452-14
acute oral, quail	PT in pollen	no effect>10,000ppm	431452-11
Manduca sexta	MT	no effect	439995-11
parasitic wasp	MT	no effect>10,000x levels found in pollen and nectar	431452-08
adult honey bee	MT	no effect>10,000x levels found in pollen and nectar	431452-07
honey bee larvae	MT	no effect>10,000x levels found in pollen and nectar	431452-06
ladybird beetle	MT	no effect>10,000x levels found in pollen and nectar	431452-09
green lacewing larvae	MT	no effect>10,000x levels found in pollen and nectar	431452-10
green lacewing larvae	MT	no effect>20ppm	434680-03
collembola	PT	no effect>8.0 g/kg	439995-12, -63
collembola	MT	no effect>0.1mg/kg	439416-01

* SDS-PAGE, Western blot, N-terminal amino acid sequencing, glycosylation, and bioactivity

Table C. Studies submitted to and reviewed by USEPA in support of registration of Cry3A products

Assay	Toxin derived from Microbes (MT) or Plants (PT)	Results	USEPA MRID Number
microbial and plant toxin equivalence (*)	MT expressed in E.coli + PT	MT and PT are equivalent (6432)	429322-03, -04, -05, and -06
acute oral, mice	MT	no effect>5220 mg/kg	429322-17
digestibility		degraded by gastric fluid but not intestinal fluid	429322-18
acute oral, quail	PT in diet	no effect> 50,000 ppm	429322-14 429322-15
parasitic wasp	PT	no effect	429322-11
honey bee larvae	MT	no effect>100ppm	441247-02
honey bee larvae	PT	non- toxic	429322-09
ladybird beetle	PT	no effect	429322-12
green lacewing larvae	PT	no effect	429322-13
collembola, 2 species	MT	no effect at 200ppm	439416-01
earthworm	MT	no effect>100mg/kg soil	441247-01

* SDS-PAGE, Western blot, N-terminal amino acid sequencing, glycosylation, and bioactivity

Table D. Studies submitted to and reviewed by USEPA in support of registration of Cry9C products

Assay	Toxin derived from Microbes (MT) or Plants (PT)	Results	USEPA MRID Number
microbial and plant toxin equivalence (*)	MT expressed in E.coli + PT	MT and PT are equivalent (6466)	443844-01
acute oral, mice	MT	no effect>3760 mg/kg	442581-07
digestibility + heat stability	MT	not degraded by gastric fluid not degraded by heat (90°C-10min)	442581-08
homology		no homology found with allergenic protein sequences in SWISS database	442581-09 443844-04
acute oral, quail	PT	no effect>58ug/l diet	442581-14
honey bee, adult	PT in pollen	no effect>5.8ug/l diet	443843-02
ladybird beetle	PT in pollen	no effect>0.36ug/l diet	442581-11
daphnia	PT in pollen	no effect>0.36ug/l diet	442581-12
collembola	MT	no effect>20gm/kg soil	442581-10
collembola	PT	no effect>180mg/kg soil	442581-10
earthworm	PT	no effect>1.84mg/kg soil	442581-13
non-target beneficial insect field study	PT	over 3 years, no differences in numbers and types of insects in Bt and non-Bt fields	442581-15
host range insect studies	MT, PT, PT in pollen	susceptible to Cry9C: European Corn Borer, tobacco budworm,and diamondback moth; non-susceptible: corn earworm	442581-06

* SDS-PAGE, Western blot, N-terminal amino acid sequencing, glycosylation, and bioactivity

Table E. Studies submitted to and reviewed by USEPA in support of registration of Cry1F products

Assay	Toxin derived from Microbes (MT) or Plants (PT)	Results	USEPA MRID Number
microbial and plant toxin equivalence (*)	MT expressed in E.coli + PT	MT and PT are equivalent	450201-03 447149-03
acute oral, mice	MT	no effect>5050 mg/kg	446911-01 450201-18
digestibility	MT	degraded by pepsin	447149-03
glycosylation	MT + PT	No glycosylation	447149-03
heat stability	MT	heat labile at and above 75 C	452748-01
amino acid sequence similarity to known allergens		no amino acid homology at a level of 8 contiguous amino acids exists for Cry1F and known allergens	449717-01
acute oral, quail	PT in corn meal	no effect>100,000ppm	450201-12
parasitic wasp	PT in pollen	no effect>320 ppm, 10x levels found in pollen	450201-11 453078-03
honey bee larvae	PT in pollen	LC50>640ng/larvae through development into adults	450415-03 453078-05
ladybird beetle	PT in pollen	no effect>480 ppm, 15x levels found in pollen	450201-10 453078-02
green lacewing larvae	PT in pollen	no effect>480 ppm, 15x levels found in pollen	450201-09 453078-01
collembola	MT	no effect>12.5 mg/kg soil	450201-07
Daphnia magna	PT in pollen	no effect>100mg/l	450201-08
earthworm	MT	no effect>2.26 mg/kg dry soil	450201-06 453078-04
monarch larvae	PT in pollen	LC50>10,000ng/ml no effect<10,000ng/ml, some growth inhibition seen at highest dose tested	451311-02
Field survey: ladybird beetles, predacious carabids, brown and green lacewings, minute pirate bugs, assassin bugs, damsel bugs, parasitic wasps, damselflies, dragonflies, and spiders.	PT	visual counts showed no significant differences except for greater numbers in Bt maize of lady beetles, pirate bugs and spiders than seen in non-transgenic lines sticky trap counts showed no significant differences except for greater numbers in Bt maize of parasitic wasps and pirate bugs	450201-13

* SDS-PAGE, Western blot, ELISA, N-terminal amino acid sequencing, glycosylation,and bioactivity

Table F. Studies submitted to and reviewed by USEPA in support of registration of Cry2Ab2 products

Assay	Toxin derived from Microbes (MT) or Plants (PT)	Results	USEPA MRID Number
microbial and plant toxin equivalence (*)	MT expressed in E.coli + PT	MT and PT are equivalent	449993-01 449394-03
acute oral, mice	MT	no effect>1450 mg/kg	449666-02
digestibility	MT	degraded by simulated gastric acid	449666-03
amino acid sequence similarity to known allergens and heat stability		no amino acid homology at a level of 8 contiguous amino acids exists for Cry2Ab2 and known allergens; heat labile at and above 120 C	449666-04 449666-05 442353-04
acute oral, quail	PT	no effect>100,000ppm	450863-16
freshwater fish	PT cottonseed meal	dietary LC50 of Bt cottonseed meal>20% of diet	450863-18 453371-03
honey bee adult and larvae	MT	no effect>100mg/ml larvae through development into adults	453371-02 450863-07 450863-08
ladybird beetle	MT	no effect>4500 ppm	450863-11
green lacewing larvae	MT	no effect>1100 ppm, 21.6x levels found in cotton	450863-09
collembola	PT cotton leaf tissue	no effect>69.5 mg/g diet	450863-14
earthworm	MT	no effect>330mg/kg dry soil	450863-13

* SDS-PAGE, Western blot, glycosylation,and bioactivity

Table G. Studies submitted to and reviewed by USEPA in support of registration of Cry3Bb1 products

Assay	Toxin derived from Microbes (MT) or Plants (PT)	Results	USEPA MRID Number
microbial and plant toxin equivalence (*)	MT expressed in E.coli + PT	MT and PT are equivalent	451568-03 454240-04 454240-05 454240-10 454240-11 455382-01
acute oral, mice	MT	no effect>2980 mg/kg	449043-06
acute oral, mice	MT	no effect>3200 mg/kg	455382-02
acute oral, mice	MT	no effect>3780 mg/kg	449043-05
gastric digestibility	MT + PT	degraded by simulated gastric fluid	449043-07 454240-06 455382-03
intestinal digestibility	MT	degraded by simulated intestinal fluid to a smaller substance which was not degraded further (Cry proteins are general resistant to trypsin)	455770-02
heat stability	PT	heat labile at and above 240 C	454240-07
amino acid sequence similarity to known allergens		no amino acid homology at a level of 8 contiguous amino acids exists for Cry2Ab2 and known allergens	449043-09 454240-08
amino acid sequence similarity to known protein toxins		no amino acid homology for Cry2Ab2 and known protein toxins	449043-08
acute oral, quail	PT maize grain	no effect>70,000ppm	449043-15
freshwater fish	PT maize grain	dietary LC50 of Bt maize>35% of diet	449043-19
Daphnia magna	PT in pollen	no effect>120mg pollen/l	449043-18
parasitic wasp larvae	MT	no effect>400 ppm	449043-13
honey bee larvae	MT	LC50>1,790 ppm - larvae through development into adults	449043-10
honey bee adults	MT	LC50>360ug/ml (20X concentration in pollen)	449043-11
green lacewing larvae	MT	LC50>8,000 ppm, 20x field exposure	449043-12
adult ladybird beetle	MT	LC50>8,000 ppm, 20x levels found in plants	449043-14
ladybird beetle larvae pollen feeding	PT in pollen	LC50>93ug/gm pollen, larvae through development into adults	455382-04
ladybird beetle adult pollen feeding	PT in pollen	no effect – 50% pollen feeding *C. maculata*	453613-01
ladybird beetle adult pollen feeding	PT in pollen	no effect – 50% pollen feeding *H. convergens*	453613-02
chronic dietary collembola	PT in leaf tissue	LC50>872.5ug (50% maize leaves in diet)	449043-17
earthworm	MT	LC50>570mg/kg dry soil	449043-16
earthworm	MT	LC50>166.6mg/kg dry soil	457571-01
monarch larvae pollen feeding	PT in pollen	no acute toxicity or developmental effects	455382-05
insecticidal activity spectrum bioassays	MT	of 6 Coleoptera Families and 2 Lepidoptera species, only 2 beetle species of one Coleoptera Family affected (corn rootworm and Colorado potato beetle)	455328-07
two year field survey		no overall differences in abundance of non-target invertebrates and less impact on beneficial insects than traditional insecticides	455382-06 457916-01

* SDS-PAGE, Western blot, ELISA, N-terminal amino acid sequencing, MALDI-TOF analysis of protein digests, glycosylation,and bioactivity

Table H. Studies submitted to and reviewed by USEPA in support of registration of Cry34Ab1/Cry35Ab1 products

Assay	Toxin derived from microbes or plants	Results	USEPA MRID Number
microbial & plant toxin equivalence (*)	MT expressed in *Pseudomonas fluorescens*	MT & PT are equivalent	461239-05 461239-06
acute oral, mice	MT, Cry34Ab1 alone	no effect>2700 mg/kg pure protein	452422-07
acute oral, mice	MT, Cry35Ab1 alone	no effect>1850mg/kg pure protein	452422-08
acute oral, mice	MT, Cry34Ab1/Cry35Ab1 mixture	no effect>482 and 1520 mg/kg of Cry34Ab1 and Cry35Ab1 pure proteins respectively	452422-09
gastric digestibility	MT	Cry34Ab1 and Cry35Ab1 degraded by simulated gastric fluid	452422-12 455845-02
heat stability	MT	mixture of Cry34Ab1 and Cry35Ab1 proteins is deactivated after exposure to 60°C, 75°C and 90°C for 30 minutes	453584-01 455845-01 458086-01 458602-01
amino acid sequence similarity to known allergens		no amino acid homology at a level of 8 contiguous amino acids exists for Cry34Ab1 and Cry35Ab1 with known allergens	452422-05
amino acid sequence similarity to known protein toxins		no amino acid homology for Cry34Ab1 and Cry35Ab1 with know protein toxins	465847-01
freshwater fish	MT, Cry34Ab1/Cry35Ab1 mixture(**)	8-d acute toxicity NOEC>100mg/kg diet	457904-03
Daphnia magna	MT, Cry34Ab1/Cry35Ab1 mixture(**)	48-h acute toxicity NOEC>100µg/mL	457904-04
parasitic wasp larvae	MT, Cry34Ab1/Cry35Ab1 mixture(**)	11-d acute toxicity NOEC>280µg/mL diet	457904-05
honey bee larvae	MT, Cry34Ab1/Cry35Ab1 mixture(**)	6-d acute toxicity NOEC>5.6µg/larva	453407-01
green lacewing larvae	MT, Cry34Ab1/Cry35Ab1 mixture(**)	10-d acute toxicity NOEC>280µg/g diet	457904-07
adult convergent ladybird beetle	MT, Cry34Ab1/Cry35Ab1 mixture(**)	11-d acute toxicity NOEC>280µg/mL diet	452422-10
twelvespotted ladybird beetle larvae Pollen feeding	PT in pollen	7-d acute toxicity, weight reduction NOEC>58.52µg/g diet	461239-12
chronic dietary collembola	MT, Cry34Ab1/Cry35Ab1 mixture(**)	28-d acute toxicity, reproduction NOEC>12.7mg/kg diet	457904-06
earthworm	MT, Cry34Ab1/Cry35Ab1 mixture(**)	7- and 14-d acute toxicity NOEC>76mg/kg dry soil	453602-01
poultry feeding	PT, grain in diet	42-day feeding study no diet-related effects	461239-11
insecticidal activity spectrum bioassay	MT, Cry34Ab1/Cry35Ab1 mixture	insects from three orders (Lepidoptera, Homoptera and Coleoptera) and four families (Pyralidae, Chrysomelidae, Aphididae and Noctuidae) were tested and only larvae of *Diabrotica* spp. were affected	457904-06
field survey	PT	no overall differences in abundance of non-target invertebrates	461239-14

* SDS-PAGE, Western blot, ELISA, N-terminal amino acid sequencing, MALDI-TOF MS peptide mass fingerprinting, glycosylation and bioactivity.

** NOECs for a Cry34Ab1/Cry35Ab1 mixture are expressed as the sum of the Cry34Ab1 and Cry35Ab1 protein concentrations.

MRID reports references for tables A - H

429322-03: Bartnicki, D., P. Lavrik, R. Leimgruber, *et al.* 1993. Equivalence of Microbially-Produced and Plant-Produced *B.t.t.* Protein also Called Colorado Potato Beetle Active Protein from *Bacillus thuringiensis* subsp. *tenebrionis*: Lab Project No. 92-01-37-07: 93-081E: 12897. Unpublished study prepared by Monsanto Co. 95 p.

429322-04: Lavrik, P. 1993. Characterization of Colorado Potato Beetle Active *Bacillus thuringiensis* subsp. *tenebrionis* Protein Produced in *Escherichia coli*: Lab Project Number: 92-01-37-10: 93-081E: 92-448-711. Unpublished study prepared by Monsanto Co. 82 p.

429322-05: Bartnicki, D., R. Leimgruber, P. Lavrik, *et al.* 1993. Characterization of the Major Tryptic Fragment from Colorado Potato Beetle Active Protein from *Bacillus thuringiensis* subsp. *tenebrionis* (*B.t.t*): Lab Project Number: 92-01-37-15: 93-081E: 12994. Unpublished study prepared by Monsanto Co. 53 p.

429322-06: Rogan, G. and P. Lavrik. 1993. Compositional Comparison of Colorado Potato Beetle (CPB) Active *Bacillus thuringiensis* subsp. *tenebrionis* (*B.t.t.*) Protein Produced in CPB Resistant Potato plants and Commercial Microbial Products: Lab Project Number: 92--01-37-17: 93-081E: 12988. Unpublished study prepared by Monsanto Co. 46 p.

429322-09: Maggi, V. 1993. Evaluation of the Dietary Effect(s) of Purified *B.t.t*. Protein on Honey Bee Larvae: Lab Project Number: CAR 188-92: 92-01-37-03: 92-448-702. Unpublished study prepared by California Agricultural Research, Inc. 52 p.

429322-11: Hoxter, K. and G. Smith. 1993. *B.t.t*. Protein: A Dietary Toxicity Study with Parasitic Hymenoptera (*Nasonia vitripennis*): Lab Project Number: 139-349B: WL-92-436: 93-081E. Unpublished study prepared by Wildlife International Ltd. 51 p.

429322-12: Hoxter, K. and G. Smith. 1993. *B.t.t*. Protein: A Dietary toxicity Study with Ladybird Beetles (*Hippodamia convergens*): Lab Project Number: 139-348: WL-92-435: 93-081E. Unpublished study prepared by Wildlife International Ltd. 47 p

429322-13: Hoxter, K. and G. Smith. 1993. *B.t.t*. Protein: A Dietary Toxicity Study with Green Lacewing Larvae (*Chrysopa carnea*): Lab Project Number: 139-347: WL-92-437. Unpublished study prepared by Wildlife International Ltd. 43 p.

429322-14: Cambell, S. J. Beavers, and M. Jaber. 1993. A Dietary Toxicity Study with Russet Burbank Potatoes in the Northern Bobwhite: Lab Project Number: 139-356: WL-93-11: 93-081E. Unpublished study prepared by Wildlife International Ltd. 87 p.

429322-15: Campbell, S. J. Beavers, and M. Jaber. 1993. A Dietary Toxicity Study with Russet Burbank Potatoes in the Northern Bobwhite: Lab Project Number: 139-357: WL-93-12. Unpublished study prepared by Wildlife International Ltd. 50 p.

429322-17: Naylor, M. 1993. Acute Oral Toxicity Study of *B.t.t*. Protein in Albino Mice: Lab Project Number: 92170: ML-92-407. Unpublished study prepared by Monsanto Co. 191 p.

429322-18: Keck, P. S. Sims, and D. Bartnicki. 1993. Assessment of the Metabolic Degradation of the Colorado Potato Beetle (CPB) Active Protein in Simulated Mammalian Digestive Models: Lab Project Number: 92-01-37-16: 93-081E. Unpublished study prepared by Monsanto Co. 145 p.

431452-02: Sammons, D. 1994. Assessment of Equivalence Between *E. coli*-Produced and Cotton-Produced *B.t.k*. HD-73 Protein and Characterization of the Cotton-Produced B.t.k. HD-73 Protein: Lab Project Number: 92/01/36/14: 13170. Unpublished study prepared by Monsanto Agricultural Group. 45 p.

431452-06: Maggi, V. 1993. Evaluation of the Dietary Effect(s) of Purified *B.t.k*. Endotoxin Proteins on Honey Bee Larvae: Lab Project Number: CAR/180/92: 92/01/36/10: 92/427/709. Unpublished study prepared by California Agricultural Research, Inc. 76 p.

431452-07: Maggi, V. 1993. Evaluation of the Dietary Effect(s) of Purified *B.t.k*. Endotoxin Proteins on Honey Bee Adults: Lab Project Number: 181/92: 92/01/36/10: 92/427/708. Unpublished study prepared by California Agricultural Research, Inc. 102 p.

431452-08: Palmer, S. and J. Beavers. 1993. *B.t.k.* HD-73 Protein: A Dietary Toxicity Study with Parasitic Hymenoptera (*Nasonia vitripennis*): Lab Project Number: 139/369: 93/234: WL/93/234. Unpublished study prepared by Wildlife International Ltd. 108 p.

431452-09: Palmer, S. and J. Beavers. 1993. *B.t.k.* HD-73 Protein: A Dietary Toxicity Study with Ladybird Beetles (*Hippodamia convergens*): Lab Project Number: 139/370: WL/93/232: 92/01/36/25. Unpublished study prepared by Wildlife International Ltd. 104 p.

431452-10: Palmer, S. and J. Beavers. 1993. *B.t.k.* HD-73 Protein: A Dietary Toxicity Study with Green Lacewing Larvae (*Chrysopa carnea*): Lab Project Number: 139/371: WL/93/233: 92/01/36/26. Unpublished study prepared by Wildlife International Ltd. 100 p.

431452-11: Cambell, S. and J. Beavers. 1993. A Dietary Toxicity Study with Cotton Seed Meal in the Northern Bobwhite: Lab Proj. No.: 139/358: WL/93/13. Unpublished study prepared by Wildlife International Ltd. 44 p.

431452-13: Naylor, M. 1993. Acute Oral Toxicity of *Bacillus thuringiensis* var. *kurstaki* (Cry IA(c)) HD-73 Protein in Albino Mice: Lab Proj. No.:92197: ML/92/493 Unpublished study prepared by Monsanto Agr. Group. 187 p.

431452-14: Ream, J. 1994. Assessment of the *In vitro* Digestive Fate of *Bacillus thuringiensis* var. *kurstaki* HD-73 Protein: Lab Proj No.: 92/01/36/22: 92/427/728. Unpublished study prepared by Monsanto Agr. Group. 43 p

433236-06: Privalle, L. 1994. *In vitro* Digestibility of CryIA(b) Protein from Bt Maize (Corn) and *Bacillus thuringiensis* subspecies *kurstaki* under Simulated Mammalian Gastric Conditions: Lab Project Number: CAB/007/94. Unpublished study prepared by Ciba Seeds Agricultural Biotechnology Research Unit. 17 p.

433236-08: Kuhn, J. 1994. Acute Oral Toxicity Study in Mice: CryIA(b) B.t.k. Delta-endotoxin: Final Report: Lab Project Number: 1238/94. Unpublished study prepared by Stillmeadow, Inc. 20 p.

433236-09: Campbell, S. 1994. CryIA(b): Enriched Maize Leaf Protein: An Acute Oral Toxicity Study with the Northern Bobwhite (*Colinus virginianus*): Lab Project Number: 108/371. Unpublished study prepared by Wildlife International Ltd. 49 p.

433236-10: Collins, M. 1994. Bt Maize Pollen (PHO176-0194): Acute Toxicity to Daphnids (*Daphnia magna*) under Static-Renewal Conditions: Lab Project Number: 94/3/5217: 1781/0394/6419/110. Unpublished study prepared by Springborn Laboratories, Inc. 59 p.

433396-01: Garvey, N. 1994. Acute (14-Day) Toxicity to Earthworms (*Eisenia foetida*) of a Lyophilized Protein Extracted from Bt-Transgenic Maize Leaves: Amended Final Report: Lab Project Number: 94-5-5282: 1781/0494/6440/630. Unpublished study prepared by Springborn Labs, Inc. 60 p.

433396-02: Obrycki, J. 1994. Feeding, Development, and Survival of the Lady Beetle Predator *Coleomegilla maculata* (Coleoptera:coccinellidae) on Transgenic "Bt" Maize (Corn) Pollen: Final Report: Lab Proj No.: PHO176/0194 ISU. Unpublished study prepared by Iowa State University, Department of Entomology. 62 p.

433972-02: Meeusen, R. and I. Mettler. 1994. Equivalence of Plant and Microbially Produced *Bacillus thuringiensis kurstaki* HD-1 Protein: Lab Project Number: 1/NK5EQ. Unpublished study prepared by Nothrup King Co.; University of Wisconsin; and Kendrick Labs. 43 p.

434157-03: Maggi, V. 1994. Evaluation of the Dietary Effect(s) of Transgenic Bt Maize (Corn) Pollen (Sample PH0176-0294) on Honeybee Development: Lab Project Number: CAR 176-94. Unpublished study prepared by California Agricultural Research, Inc. 57 p.

434175-02: Shapiro, R. 1993. EPA Acute Dermal Toxicity Limit Test (in Rabbits): PHT 93-003: Lab Project Number: T-2414. Unpublished study prepared by Product Safety Labs. 15 p.

434392-01: Ream, J. 1994. Assessment of the *In vitro* Digestive Fate of *Bacillus thuringiensis* subsp. *kurstaki* HD-1 Protein: Lab Project Number: 93-01-39-04. Unpublished study prepared by Monsanto Co. 44 p.

434392-02: Maggi, V.and S. Sims. 1994. Evaluation of the Dietary Effects of Purified B.t.k. Endotoxin Proteins on Honey Bee Larvae: Lab Project Number: IRC-91-ANA-13. Unpublished study prepared by Monsanto Co. 51 p.

434392-03: Maggi, V.and S. Sims. 1994. Evaluation of the Dietary Effects of Purified B.t.k. Endotoxin Proteins on Honey Bee Adults: Lab Project Number: IRC-91-ANA-12. Unpublished study prepared by Monsanto Co. 70 p.

434635-01: Collins, M. 1994. Bt Maize (Corn) Leaf Protein (LP176-0194)- 28-Day Survival and Reproduction Study in Collembola (*Folsomia candida*): Final Report: Lab Project Number: 94/10/5523: 1781/0494/6444/123. Unpublished study prepared by Springborn Labs, Inc. 92 p.

434680-01: Naylor, M. 1992. Acute Oral Toxicity Study of Btk HD-1 Tryptic Core Protein in Albino Mice: Lab Project Numbers: 92069: 11985: ML92069. Unpublished study prepared by Monsanto Co. 264 p.

434680-05: Hoxter, K.and S. Lynn. 1992. Activated Btk HD-1 Protein: A Dietary Toxicity Study With Ladybird Beetles: Lab Project Numbers: WL-92-156: 139-321. Unpublished study prepared by Monsanto Co.; and Wildlife Int'l, Ltd. 23 p.

434680-03: Hoxter, K.and S. Lynn. 1992. Activated Btk HD-1 Protein: A Dietary Toxicity Study With Green Lacewing Larvae: Lab Project Numbers: WL-92-155: 139-3388. Unpublished study prepared by Monsanto Co.; and Wildlife Int'l, Ltd. 21 p.

435332-03: Lee, T.; M. Bailey, and P. Sanders. 1995. Compositional Comparison of *Bacillus thuringiensis* subsp. *kurstaki* HD-1 Protein Produced in European Corn Borer Resistant Corn and the Commercial Microbial Product, DIPEL: Lab Proj No.: 94-01-39-12: MSL 13876. Unpublished study prepared by Monsanto Co. 35 p.

435332-05: Campbell, S. and J. Beavers. 1994. A Dietary Toxicity Study with MON 80187 Meal in the Northern Bobwhite: Lab Proj No.: WL 94-150: 139-387. Unpublished study prepared by Wildlife International Ltd. 32 p.

438879-01: Jackson, L., E. Robinson, D. Nida, *et al*. 1995. Evaluation of the European Corn Borer Resistant Corn Line MON 801 as a Feed Ingredient for Catfish: Lab Project Number: 94-01-39-16: 14066: 95-459-720. Unpublished study prepared by Mississippi State University Delta Research and Extension Center. 39 p.

438879-02: Palmer, S.and J. Beavers. 1995. CRYIA(b) Insecticidal Protein: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate: Final Report: Lab Project Number: 139-417: WL-95-281. Unpublished study prepared by Wildlife International Ltd. 25 p.

439416-01: Sims, S.and J. Martin. 1996. Effect of the *Bacillus thuringiensis* Insecticidal Proteins CryIA(b), CryIA(c), CryIIA, and CryIIIA on *Folsomia candida* and *Xenylla grisea* (Insecta: Collembola): Lab Project Number: 93-081E1. Unpublished study prepared by Monsanto Co. 22 p.

439995-01: Merriman, T. 1996. An Acute Oral Toxicity Study in Mice with *Bacillus thuringiensis* subsp. *kurstaki* CryIA(c) Delta Endotoxin: Lab Project Number: DGC-95-A17: 3406.1: S95.001.3406. Unpublished study prepared by DEKALB Genetics Corp. and Springborn Laboratories (SLI). 52 p.

439995-03: Walters, D.and W. Adams. 1996. *In vitro* Digestibility of CryIA(c) and (inert ingredient): Lab Project Number: DGC-96-A22. Unpublished study prepared by DEKALB Genetics Corp. 16 p.

439995-11: Kruger, D. and D. Walters. 1996. Evaluation of the Pollen Expression of CryIA(c) Insecticidal Protein in DEKALB Transformant DBT418 Using Manduca sexta Toxicity Assays: Lab Project Number: DGC-95-A21. Unpublished study prepared by DEKALB Genetics Corp. and Wildlife Int'l. Ltd. 43 p.

439995-12: Collins, M. 1996. Transgenic Maize Leaf Tissue and Microbially Produced CryIA(c) Protein: Chronic Toxicity To Collembola (*Folsomia candida*), Under Static Conditions: Lab Project Number: DGC-95-A16: 13601.1195.6101.123: 96-3-6408. Unpublished study prepared by DEKALB Genetics Corp. and Springborn Laboratories, Inc. (SLI). 141 p.

441247-01: Palmer, S. and J. Beavers. 1996. *Bacillus thuringiensis* subsp. *tenebrionis* (*B.t.t.*) Protein: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate: Lab Project Number: 139-416: WL-96-151: 96-01-37-01. Unpublished study prepared by Wildlife International Ltd. 54 p.

441247-02: Maggi, V. 1996. Evaluation of the Dietary Effect(s) of Purified *B.t.t*. Protein on Honey Bee Larvae: Lab Project Number: CAR 111-96: XX-96-088: 96-01-37-01. Unpublished study prepared by California Ag Research Inc. 69 p.

442353-04: Astwood, J. 1996. *Bacillus thuringiensis* subsp. *kurstaki* P2A Insecticidal Protein (CryIIA Protein) Shares No Significant Sequence Similarity with Proteins Associated with Allergy or Coeliac Disease: Lab Project Number: 14630. Unpublished study prepared by Monsanto Co. 71

442581-06: Halliday, W. 1997. Insect Host Range Comparison of Cry9C Protein: Lab Project Number: 7094-96-0290-AC-001: 7094: 7094-96-0290-AC. Unpublished study prepared by Ricerca, Inc. 85 p.

442581-07: Douds, D. 1997. An Acute Oral Toxicity Study in Mice with Cry9C Protein as Purified from *Bacillus thuringiensis* Cry9C.PGS2: Final Report: Lab Project Number: 3433.1. Unpublished study prepared by Springborn Labs, Inc. 44 p.

442581-08: Peferoen, M. 1997. *In vitro* Digestibility and Heat Stability of the Endotoxin Cry9C and *Bacillus thuringiensis*: (Final Report). Unpublished study prepared by Plant Genetic Systems N.V. 13 p.

442581-09: MacIntosh, S. 1997. Amino Acid Sequence Homology Search with the Corn Expressed Truncated Cry9C Protein Sequence. Unpublished study prepared by Plant Genetic Systems N.V. 238 p.

442581-10: Halliday, W. 1996. Chronic Exposure of *Folsomia candida* to Corn Tissue or Bacteria Expressing Cry9C Protein: (Final Report): Lab Project Number: 6913-96-0158-AC-001: 6913: 6913-96-0158-AC-000. Unpublished study prepared by Ricerca, Inc. 65 p.

442581-11: Palmer, S. and J. Beavers. 1997. Cry9C Protein in Corn Pollen: A Dietary Toxicity Study with the Ladybird Beetle (*Hippodamia convergens*): (Final Report): Lab Proj No. 452-101: 452/102496/LBLC/SUB452. Unpublished study prepared by Wildlife International Ltd. 36 p. {OPPTS 885-4350}.

442581-12: Graves, W. and J. Swigert. 1997. Cry9C Protein in Corn Pollen: A 48-Hour Static-Renewal Acute Toxicity Test with the Cladoceran (*Daphnia magna*): Final Report: Lab Project Number: 452A-101. Unpublished study prepared by Wildlife International Ltd. 23 p.

442581-13: Palmer, S.and J. Beavers. 1997. Cry9C Protein in Plant Powder: An Acute Toxicity Test with the Earthworm in an Artificial Soil Substrate: (Final Report): Lab Project Number: 452-103: 452/102496/EWSDT.WCA/SUB452. Unpublished study prepared by Wildlife International Ltd. 39 p

442581-14: Palmer, S., B. Grimes, and J. Beavers. 1997. Corn Plant Powder Containing Cry9C Protein: A Dietary Toxicity Study with the Northern Bobwhite: (Final Report): Lab Project Number: 452-102: 452/102496/QLC-LIM/SUB452. Unpublished study prepared by Wildlife International Ltd. 47 p.

442581-15: MacIntosh, S. 1997. Effects of Cry9C Corn on Predatory Non-Target Beneficial Insects and Endangered Species: Lab Proj No. 96QZM004. Unpublished study prepared by Plant Genet. Systems N.V. 48 p.

442715-01: Halliday, W. 1997. Chronic Exposure of *Folsomia candida* to Corn Tissue Expressing CryIA(B) Protein: Lab Project Number: 7140-97-0030-AC-001: XX-97-064: 95-152E2. Unpublished study prepared by Ricerca, Inc. 91 p.

443843-02: Palmer, S. and H. Krueger. 1997. Cry9C Protein in Corn Pollen: A Dietary Toxicity Study with the Honey Bee (*Apis mellifera*): Lab Project Number: 452-104: 452/060997/BLCP/SUB452. Unpublished study prepared by Wildlife International Ltd. 38 p. {OPPTS 885.4380}

443844-04:vPeferoen, M. 1997. Cry9C *Bacillus thuringiensis* Insecticidal Protein Identification of Sequence Homology with Allergens by Searching Protein Databanks. Unpublished study prepared by Plant Genetic Systems NV. 10 p.

443844-01: Peferoen, M. 1997. Determination of Test Substance Equivalence Between Corn Plant Produced Cry9C and Bacterial Produced Cry9C: Interim Report: Lab Project Number: 96QZM007. Unpublished study prepared by Plant Genetic Systems NV. 20 p.

446911-01: Kuhn, J. 1998. Acute Oral Toxicity Study in Mice: Cry 1F *Bacillus thuringiensis* subsp. *aizawai* Delta-endotoxin: Final Report: Lab Project Number: 4281-98. Unpublished study prepared by Stillmeadow Inc. 11 p.

447149-03: Irrig, H. 1998. Characterization Report of CGA-293343 5FS: (A-9765 C): Lab Project Number: 732-98. Unpublished study prepared by Novartis Crop Protection, Inc. 14 p.

449043-05: Bechtel, C. 1999. Acute Oral Toxicity Study of *B.t.* Protein 11231 in Mice: Lab Project Number: 16216: ML-98-142: MSE-N 98036. Unpublished study prepared by Monsanto Company. 212 p.

449043-06, Bechtel, C. 1999. Acute Oral Toxicity Study of *B.t.* Protein 11098 in Mice: Lab Project Number: 16215: ML-98-141: MSE-N 98035. Unpublished study prepared by Monsanto Company. 212 p.

449043-07: Leach, J., R. Hileman, J. Martin, *et al*. 1999. Assessment of the *in vitro* Digestibility of *B.t.* Protein 11098 and *B.t.* Protein 11231 Utilizing Mammalian Digestive Fate Models: Lab Project Number: 98-01-39-02: 15704. Unpublished study prepared by Monsanto Company. 40 p.

449043-08: Astwood, J. and R. Hileman. 1999. Bioinformatics Analysis of *B.t.* Protein 11098 and *B.t.* Protein 11231 Sequences Utilizing Toxin and Public Domain Genetic Databases: Lab Project Number: 15870. Unpublished study prepared by Monsanto Company. 294 p.

449043-09: Astwood, J. and R. Hileman. 1999. Bioinformatics Analysis of *B.t.* Protein 11098 and *B.t.* Protein 11231 Sequences Utilizing an Allergen Database: Lab Project Number: 15873. Unpublished study prepared by Monsanto Company. 38 p.

449043-10: Maggi, V. 1999. Evaluation of the Dietary Effects of Purified *Bacillus thuringiensis* Protein 11231 on Honey Bee Larvae: Lab Project Number: CA-98-169: CAR 158-98. Unpublished study prepared by Monsanto Company, and California Agricultural Research, Inc. 46 p.

449043-11: Maggi, V. 1999. Evaluation of the Dietary Effects of Purified *Bacillus thuringiensis* Protein 11231 on Adult Honey Bees (*Apis mellifera* L.): Lab Project Number: CA-98-171: CAR 160-98: 16169. Unpublished study prepared by Monsanto Company and California Agricultural Research, Inc. 58 p. {OPPTS 885.4380}

449043-12: Palmer, S. and H. Krueger. 1999. *Bacillus thuringiensis* Protein 11231: A Dietary Toxicity Study with Green Lacewing Larvae (*Chrysoperla carnea*): Lab Project Number: WL-98-298: 16165. Unpublished study prepared by Monsanto Company and Wildlife International Ltd. 40 p.

449043-13: Palmer, S. and H. Krueger. 1999. *Bacillus thuringiensis* Protein 11231: A Dietary Toxicity Study with the Parasitic Hymenoptera (*Nasonia vitripennis*): Lab Project Number: WL-98-300: 16167. Unpublished study prepared by Monsanto Company and Wildlife International Ltd. 50 p.

449043-14: Palmer, S. and H. Krueger. 1999. *Bacillus thuringiensis* Protein 11231: A Dietary Toxicity Study with the Ladybird Beetle (*Hippodamia convergens*): Lab Project Number: WL-98-299: 16166. Unpublished study prepared by Monsanto Company and Wildlife International Ltd. 41 p.

449043-15: Gallagher, S., J. Grimes, J. Beavers. 1999. *Bacillus thuringiensis* Protein 11231 in Corn Grain: A Dietary Toxicity Study with the Northern Bobwhite: Lab Project Number: WL-99-014: 16161: 139-44. Unpublished study prepared by Monsanto Company and Wildlife International Ltd. 43 p.

449043-16: Hoxter, K., S. Palmer, and H. Krueger. 1999. *Bacillus thuringiensis* Protein 11231: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate: Lab Project Number: WL-99-013: 16162: 139-443A. Unpublished study prepared by Monsanto Company and Wildlife International Ltd. 45 p.

449043-17: Teixeira, D. 1999. Assessment of Chronic Toxicity of Corn Tissue Containing the *Bacillus thuringiensis* Protein 11098 to Collembola (*Folsomia candida*): Lab Project Number: SB-98-296: 15988: 252.6149. Unpublished study prepared by Monsanto Company and Springborn Labs. 55 p.

449043-18: Drottar, K. and H. Krueger. 1999. *Bacillus thuringiensis* Protein 11098 in Corn Pollen: A 48-Hour Static-Renewal Acute Toxicity with the Cladoceran (*Daphnia magna*): Lab Project Number: WL-98-295: 16163: 139A-236. Unpublished study prepared by Monsanto Company and Wildlife International Ltd. 31 p.

449043-19: Meng, L. and E. Robinson. 1999. Evaluation of Insect Protected Corn Lines MON 853 and MON 859 as a Feed Ingredient for Catfish: Lab Project Number: XX-98-297: 16164. Unpublished study prepared by Monsanto Company and Mississippi State University. 32 p.

449394-03: Holleschak, G., T. Lee, J. Astwood, *et al*. 1999. Assessment of the Equivalence of Proteins Expressed in Cotton Events 15813 and 15985: Lab Project Number: 99-01-36-06: MSL-16093: 99-55E. Unpublished study prepared by Monsanto. 78 p.

449666-02: Bechtel, C. 1999. Acute Oral Toxicity Study of Insect Protection Protein 2 (IPP2) in Mice: Lab Project Number: MSE-N 98072: ML-98-304: 16381. Unpublished study prepared by Monsanto Company, Metabolism and Safety Evaluation-Newstead, and Monsanto Company, Protein Characterization and Safety Center. 209 p.

449666-03: Leach, J., R. Hileman, J. Martin, *et al*. 1999. Assessment of the *in vitro* Digestibility of Insect Protection Protein 2 (IPP2) Utilizing Mammalian Digestive Fate Models: Lab Project Number: 98-01-39-04: MSL 16046: MONSANTO 99-819E. Unpublished study prepared by Monsanto Company. 33 p.

449666-04: Astwood, J.and R. Hileman. 1999. Bioinformatics Analysis of Insect Protection Protein 2 (IPP2) Sequence Utilizing an Allergen Database: Lab Project Number: 16094: 99-01-62-09: MONSANTO 99-819E. Unpublished study prepared by Monsanto Company. 36 p.

449666-05: Astwood, J., R. Hileman. 1999. Bioinformatics Analysis of Insect Protection Protein 2 (IPP2) Sequence Utilizing Toxin and Public Domain Genetic Databases: Lab Proj No. 16095: 99-01-62-10: MONSANTO 99-819E. Unpublished study prepared by Monsanto Company. 230 p.449717-01: Meyer, T. 1999. Comparison of Amino Acid Sequence Similarity of Cry1F and PAT Proteins to Known Allergen Proteins: Final Report: Lab Proj No. PHI99-013. Unpublished study prepared by Trait and Technology Development. 24 p.

449993-01: Hileman, R. P. Pyla, and T. Lee. 1999. Characterization of Insect Protection Protein (IPP2) Produced by Fermentation: Lab Project Number: 98-01-39-16: 99-819E: 15742. Unpublished study prepared by Monsanto Company. 33 p.

450201-03: Young, D. 1999. Characterization of Expressed Cry1F Protein in Maize Tissues (Pollen, Grain, Grain-Containing Feed, and Purified Maize-Expressed Cry1F Protein) and Microbial Expressed Cry1F Delta Endotoxin by Biological and Biochemical Procedures: Lab Project Number: 990027. Unpublished study prepared by Pioneer Hi-Bred International, Inc. and Dow AgroSciences LLC. 71 p.

450201-06: Hoxter, K., J. Porch, and H. Krueger. 1999. CRY 1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate: Final Report: Lab Project Number: 354-112. Unpublished study prepared by Wildlife International, Ltd. 32 p.

450201-07: Halliday, W. 1998. Chronic Exposure of *Folsomia candida* to Bacterially Expressed Cry 1F Protein: Lab Project Number: 7335-98-0078-AC-001. Unpublished study prepared by Ricerca, Inc. 122 p.

450201-08: Drottar, K. and H. Krueger. 1999. Bt Cry 1F Delta-Endotoxin: A 48-Hour Static Renewal Acute Toxicity Test with the Cladoceran (*Daphnia magna*) using Bacterially Expressed Bt Cry 1F Delta-Endotoxin, and Pollen from Maize Expressing Bt Cry 1F Delta-Endotoxin: Final Report: Lab Project Number: 354A-111. Unpublished study prepared by Wildlife International, Ltd. 21 p.

450201-09: Hoxter, K. H. Krueger, and J. Porch. 1999. Cry 1F *Bacillus thuringiensis* aar. *aizawai* Delta-Endotoxin: A Dietary Toxicity Study with Green Lacewing Larvae: Lab Project Number: 354-115A. Unpublished study prepared by Wildlife International, Ltd. 28 p. {OPPTS 885.4340}

450201-10: Hoxter, K. H. Krueger, and J. Porch. 1999. Cry 1F *Bacillus thuringiensis* var. *aizawai* Delta-Endotoxin: A Dietary Toxicity Study with the Ladybird Beetle: Lab Project Number: 354-113B. Unpublished study prepared by Wildlife International, Ltd. 32 p. {OPPTS 885.4340}

450201-11: Hoxter, K., H. Krueger, and J. Porch. 1999. Cry 1F *Bacillus thuringiensis* var. *aizawai* Delta-Endotoxin: A Dietary Toxicity Study with Parasitic Hymenoptera: Lab Project Number: 354-114D. Unpublished study prepared by Wildlife International, Ltd. 33 p. {OPPTS 885.4340}

450201-12: Gallagher, S., J. Grimes, and J. Beavers. 1999. Transgenic Corn Expressing *Bacillus thuringiensis* var. *aizawai* (Bt) Cry1F Delta-Endotoxin: A Dietary Toxicity Study with the Northern Bobwhite: Lab Project Number: 354-116. Unpublished study prepared by Wildlife International, Ltd. 38 p.

450201-13: Higgins, L. 1999. Field Survey of Beneficial Arthropods Associated with *Bacillus thuringiensis* Cry1F Maize: Final Report: Lab Project Number: PHI99-018. Unpublished study prepared by Pioneer Hi-bred International, Inc. 20 p.

450201-18: Shanahan, D. 1999. Supplement to MRID 44691101: Supplemental Data for Acute Oral Toxicity Study in Mice: Cry1F *Bacillus thuringiensis* var. *aizawai* Delta-Endotoxin: Lab Project Number: GH-C 5033. Unpublished study prepared by Dow AgroSciences. 17 p.

450415-03: Maggi, V. 1999. Evaluation of the Dietary Effect(s) on Honeybee Development Using Bacterially Expressed Bt Cry1F Delta-Endotoxin and Pollen from Maize Expressing Bt Cry1F Delta Endotoxin: Lab

Project Number: CAR 172-99. Unpublished study prepared by California Agricultural Research, Inc. 53 p. {OPPTS 885.4380}

450863-07: Maggi, V. 2000. Evaluation of the Dietary Effect(s) of Insect Protection Protein 2 on Honey Bee Larvae: Lab Project Number: MSL 16175: CA-99-059: CAR 157-99. Unpublished study prepared by Monsanto Company. 29 p.

450863-08: Maggi, V. 2000. Evaluation of the Dietary Effect(s) of Insect Protection Protein 2 on Adult Honey Bees (*Apis mellifera* L.): Lab Project Number: 99-858E: CA-99-058: CAR 156-99. Unpublished study prepared by Monsanto Company. 35 p.

450863-09: Palmer, S. and H. Krueger. 2000. Insect Protection Protein 2: A Dietary Toxicity Study with Green Lacewing Larvae (*Chrysoperla carnea*): Lab Project Number: WL-99-061: MSL 16171: 139-447A. Unpublished study prepared by Wildlife International Ltd. 33 p.

450863-11: Palmer, S. and H. Krueger. 2000. Insect Protection Protein 2: A Dietary Toxicity Study with the LadyBird Beetle (*Hippodamia convergens*): Lab Project Number: 139-446A: WL-99-060: MSL 16172. Unpublished study prepared by Wildlife International Ltd. 40 p.

450863-13: Palmer, S. and H. Krueger. 2000. Insect Protection Protein 2: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate: Lab Project Number: 99-858E: 139-445: WL-99-067. Unpublished study prepared by Wildlife International Ltd. 38 p.

450863-14: Teixiera, D. 2000. Assessment of Chronic Toxicity of Cotton Tissue Containing Insect Protection Protein 2 to Collembola (*Folsomia candida*): Amended Final Report: Lab Project Number: 252.6152: SB-99-063: MSL 16174. Unpublished study prepared by Springborn Laboratories, Inc. 49 p.

450863-16: Gallagher, S., J. Grimes, and J. Beavers. 2000. Insect Protection Protein 2 In Cottonseed Meal: A Dietary Toxicity Study with the Northern Bobwhite: Lab Project Number: 139-449: WL-99-065: MSL 16178. Unpublished study prepared by Wildlife International, Ltd. 40 p.

450863-18: Li, M. and E. Robinson. 2000. Evaluation of Cottonseed Meal Derived from Insect Protected Cotton Lines 15813 and 15985 as a Feed Ingredient for Catfish: Final Report: Lab Proj No. XX-99-056: MSL 16179: 99-858E. Unpublished study prepared by Thad Cochran National Warmwater Aquaculture Center. 22 p.451311-02: Bystark, P. 2000. Toxicity of the Cry1F Protein to Neonate Larvae of the Monarch Butterfly (*Danaus plexippus* (Linnaeus)): Lab Project Number: GH-C 5073. Unpublished study prepared by Mycogen Seeds. 15 p.

451568-03: Hollenschak, G., R. Hileman, and J. Astwood. 2000. Assessment of the Physicochemical Equivalence of Cry3Bb1.11098 and NPTII Proteins in Corn Event MON 863 to Microbial Sources: Lab Project Number: 99-01-39-31: MSL-16596: GEN-POL-005. Unpublished study prepared by Monsanto Company. 38 p.

452422-05: Stelman, S. 2000. Comparison of the Amino Acid Sequence of the *Bacillus thuringiensis* Strain PS149B1 13.6 kDa and 43.8 kDa Insecticidal Crystal Proteins to Known Protein Allergens: Lab Project Number: GH-C 5140. Unpublished study prepared by Dow AgroSciences LLC. 188 p.

452422-07: Brooks, K. and P. DeWildt. 2000. PS149B1 14 KDA Protein: Acute Oral Toxicity Study in CD-1 Mice: Lab Project Number: 001130. Unpublished study prepared by Dow AgroSciences LLC. 39 p.

452422-08: Brooks, K. and P. DeWildt. 2000. PS149B1 44 KDA Protein: Acute Oral Toxicity Study in CD-1 Mice: Lab Project Number: 001129. Unpublished study prepared by Dow AgroSciences LLC. 39 p.

452422-09: Brooks, K. and P. DeWildt. 2000. PS149B1 14 KDA and 44 KDA Proteins: Acute Oral Toxicity Study in CD-1 Mice: Lab Project Number: 001128. Unpublished study prepared by Dow Chemical Company. 45 p. {OPPTS870.1100}

452422-10: Bryan, R., J. Porch, and H. Krueger. 2000. PS149B1 Binary Insecticidal Crystal Protein: A Dietary Toxicity Study with the Lady Bird Beetle: Final Report: Lab Project Number: 379-103. Unpublished study prepared by Wildlife International Ltd. 18 p. {OPPTS 885.4340}

452422-12: Korjagin, V. 2000. *In Vitro* Digestibility of PS149B1 Proteins: Lab Project Number: 000302. Unpublished study prepared by Dow AgroSciences LLC. 37 p.452748-01: Herman, R. 2000. Thermobility of

Cry1F (truncated) Delta-Endotoxin: Lab Project Number: GH-C 5144. Unpublished study prepared by Dow AgroSciences LLC. 13 p.

453078-01: Mayes, M. 2001. Supplement to MRID 45020109: Cry 1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin: A Dietary Toxicity Study with Green Lacewing Larvae: Lab Project Number: GH-C 5168. Unpublished study prepared by Dow AgroSciences. 6 p.

453078-02: Mayes, M. 2001. Supplement to MRID 45020110: Cry 1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin: A Dietary Toxicity Study with the Ladybird Beetle: Lab Project Number: GH-C 5169. Unpublished study prepared by Dow AgroSciences LLC. 6 p.

453078-03: Mayes, M. 2001. Supplement to MRID 45020111: Cry 1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin: A Dietary Toxicity Study with Parasitic Hymenoptera: Lab Project Number: GH-C 5170. Unpublished study prepared by Dow AgroSciences LLC. 6 p.

453078-04: Mayes, M. 2001. Supplement to MRID 45020106: Cry 1F *Bacillus thuringiensis* var. *aizawai* Delta Endotoxin: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate: Lab Project Number: GH-C 5171. Unpublished study prepared by Dow AgroSciences LLC. 8 p.

453078-05: Mayes, M. 2001. Suppl. to MRID 45041503: Evaluation of the Dietary Effect(s) on Honeybee Develop. Using Bacterially Expressed Bt Cry 1F Delta-Endotoxin and Pollen from Maize Expressing Bt Cry 1F Delta-Endotoxin: Lab Project Number: GH-C 5172. Unpublished study prepared by Dow AgroSciences LLC. 6 p.

453371-02: Maggi, V. 2000. Evaluation of the Dietary Effect(s) of Purified Bacillus thuringiensis Protein on Honey Bee Larvae: Lab Project Number: CAR 136-00: CA 2000-91: MSL-16961. Unpublished study prepared by California Agricultural Research, Inc. 33 p. {OPPTS 885.4380}

453371-03: Li, M. and E. Robinson. 2001. Evaluation of Cottonseed Meal Derived from Insect Protected Cotton Lines 15813 and 15985 as a Feed Ingredient for Catfish: Lab Project Number: XX-99-056: MSL 16179. Unpublished study prepared by Thad Cochran National Warmwater Aquaculture Center 23 p.

453407-01: Maggi, V. 2001. Microbial PS149B1 Binary Insecticidal Crystal Protein, Pollen Expressing PS149B1 Binary Insecticidal Crystal Protein, and Individual PS149B1 14 kDa and 44 kDa Insecticidal Crystal Proteins: Evaluation of Dietary Exposure on Honeybee Development: Lab Project Number: CAR 149-00. Unpublished study prepared by California Agricultural Research, Inc. 58 p. {OPPTS 885.4380}

453584-01: Herman, R. 2000. Thermolability of PS149B1 Binary Delta-Endotoxin: Lab Project Number: 001041. Unpublished study prepared by Dow AgroSciences LLC. 18 p.

453602-01: Bryan, R.; Porch, J.; Krueger, H. (2000) PS149B1 Binary Insecticidal Crystal Protein: Acute Toxicity to the Earthworm in an Artificial Substrate: Final Report: Lab Project Number: 379-104. Unpublished study prepared by Wildlife International, Ltd. 22 p.

453613-01: Duan, J., G. Head, M. McKee, *et al*. 2001. Dietary Effects of Transgenic *Bacillus thuringiensis* (Bt) Corn Pollen Expressing a Variant of Cry3Bb1 Protein on Adults of the Ladybird Beetle, *Coleomegilla maculata*: Lab Project Number: MSL-16936: 00-01-39-37. Unpublished study prepared by Monsanto, Co. 35 p.

453613-02: Bryan, R., J. Porch, and H. Krueger. 2001. Dietary Effects of Transgenic BT Corn Pollen Expressing a Variant of Cry3Bb1 Protein on Adults of the Ladybird Beetle, *Hippodamia convergens*: Final Report: Lab Proj No. WL-2000-158: MSL-17171: 139-453. Unpublished study prepared by Wildlife International, Ltd. 36 p.

454240-04: Holleschak, G., T. Lee, P. Pyla, *et al*. 2001. Amended Report for MSL-15835: Assessment of the Equivalent of *B.t.* Protein 11098, *B.t.* Protein 11231 and NPTII Protein Expressed in Corn Events MON 853 and MON 860 to Microbial Sources: Lab Project Number: 98-01-39-18: MSL-17222: 17222. Unpublished study prepared by Monsanto Co. 53 p.

454240-05: Holleschak, G., R. Hileman, and J. Astwood. 2001. Amended Report to MSL-16596: Assessment of the Physiochemical Equivalence of Cry3b1.11098 and NPTII Proteins in Corn Event MON 863 Microbial Sources: Lab Project Number: 99-01-39-31: MSL-17220: 17220. Unpublished study prepared by Monsanto Co. 40 p.

454240-06: Leach, J., R. Hileman, J. Martin, *et al*. 2001. Amended Report for MSL-15704: Assessment of the *In Vitro* Digestibility of *B.t.* Protein 11098 and *B.t.* Protein 11231 Utilizing Mammalian Digestive Fate Models: Lab Project Number: 98-01-39-02: 17166: MSL-17166. Unpublished study prepared by Monsanto Co. 41 p.

454240-07: Holleschak, G., R. Hileman, and J. Astwood. 2001. Amended Report for MSL-16597: Immuno-Detectability of Cry3Bb1.11098 and Cry3Bb1.11231 Proteins in the Grain of Insect Protected Corn Events MON 863 and MON 853 After Heat Treatment: Lab Project Number: 99-01-39-32: MSL-17223: 17223. Unpublished study prepared by Monsanto Co. 36 p.

454240-08: Hileman, R., E. Rice, R. Goodman, *et al*. 2001. Bioinformatics Evaluation of the Cry3Bb1 Protein Produced in Corn Event MON 863 Utilizing Allergen, Toxin and Public Domain Protein Databases: Lab Project Number: 01-01-39-23: 17140: MSL-17140. Unpublished study prepared by Monsanto Co. 102 p.

454240-10: Hileman, R. and J. Astwood. 2001. Additional Characterization of the Cry3Bb1 Protein Produced in Corn Event MON 863: Lab Proj No. 17137: MSL-17137. Unpublished study prepared by Monsanto Co. 22 p.

454240-11: Thoma, R., G. Holleschak, R. Hileman, *et al*. 2001. Primary Structural Protein Characterization of Corn Event MON 863 Cry3Bb1.11098 Protein Using N-Terminal Sequencing and MALDI Time of Flight Mass Spectrometric Techniques: Lab Proj No.17154: MSL-17154. Unpubl. study prepared by Monsanto Co. 21 p.

455382-01: Hileman, R., G. Holleschak, L. Turner, *et al*. 2001. Characterization and Equivalence of the Cry3Bb1 Protein Produced by *E. coli* Fermentation and Corn Event MON 863: Lab Project Number: 17274: 01-01-39-30. Unpublished study prepared by Monsanto Company. 75 p.

455382-02: Bonnette, K. and P. Pyla. 2001. An Acute Oral Toxicity Study in Mice with *E. coli* Produced Cry3Bb1.11098(Q349R) Protein: Amended Final Report: Lab Project Number: 3044.856: SB-2001-085: MSL-17382. Unpublished study prepared by Springborn Laboratories, Inc. 154 p.

455382-03: Leach, J., R. Hileman, and J. Astwood. 2001. Assessment of the *In Vitro* Digestibility of Cry3Bb1 Protein Purified from Corn Event MON 863 and Cry3Bb1 Protein Purified from *E. coli*: Lab Project Number: 17292: 01-01-39-13. Unpublished study prepared by Monsanto Company. 43 p.

455382-04: Duan, J., M. McKee, and T. Nickson. 2001. Dietary Effects of Transgenic *Bacillus thuringiensis* (Bt) Corn Pollen Expressing a Variant of Cry3Bb1 Protein on Larvae of the Ladybird Beetle, *Coleomegilla maculata*: Lab Project Number: MSL-16907: 00-01-39-26. Unpublished study prepared by Monsanto Company. 39 p.

455382-05: Sears, M. and H. Mattila. 2001. Determination of the Toxicity of Corn Pollen Expressing a Cry3Bb1 Variant Protein to First Instar Monarch Butterfly Larvae (*Danaus plexippus*) via Laboratory Bioassay: Lab Project Number: MSL-17235: 01-01-39-26. Unpublished study prepared by University of Guelph. 33 p.

455382-06: Bhatti, M., C. Pilcher, M. McKee, *et al*. 2001. Field Evaluation for the Ecological Impact of Corn Rootworm Insect-Protected Corn on Non-Target Organisms: Lab Project Number: 00-01-39-16: MSL-17179. Unpublished study prepared by Monsanto Agronomy Center, and Monsanto Company. 42 p.

455382-07: Head, G., M. Pleau, S. Sivausupramanian, et. al. (2001) Insecticidal Spectrum of Activity for Cry3Bb Protein *in vitro*: Lab Project Number: C3NTO. Unpublished study prepared by Monsanto Company. 12 p.

455770-02: Kileman, R., J. Leach, and J. Astwood. 2001. Assessment of the *In Vitro* Digestibility of the Cry3Bb1.11098 (Q349R) Protein in Simulated Intestinal Fluid: Lab Project Number: 17530: 01-01-62-11. Unpublished study prepared by Monsanto Company. 36 p.

455845-01: Herman, R. 2002. Heat Lability of Individual Proteins of the PS149B1 Binary ICP: Lab Project Number: 010144. Unpublished study prepared by Dow AgroSciences LLC. 15 p.

455845-02: Korjagin, V., R. Herman, P. Hunst, *et al*. 2002. *In Vitro* Simulated Gastric Fluid Digestibility of Microbially Derived Cry34Ab1 Protein: Lab Project Number: 010111. Unpublished study prepared by Dow AgroSciences LLC. 95 p.

457571-01: Sindermann, A., J.Porch, and H. Krueger. 2002. Evaluation of a Cry3Bb1 Protein Variant in an Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate: Lab Project Number: MSL-18137: 139-457: WL-2001-129. Unpublished study prepared by Wildlife International, Ltd. and Monsanto Company. 42 p.

457904-03: Marino, T. and A. Yaroch. 2002. PS1498B1 Binary Insectidical Crystal Protein: An 8-Day Dietary Toxicity Study With the Rainbow Trout, *Oncorhynchus mykiss*, Walbaum: Lab Project Number: 011193. Unpublished study prepared by The Dow Chemical Company. 31 p.

457904-04: Marino, T. and A. Yaroch. 2001. PS 149B1 Binary Insecticidal Crystal Protein: An Acute Toxicity Study With the Daphnid, *Daphnia magna* Straus: Lab Project Number: 011137. Unpublished study prepared by The Dow Chemical Company. 28 p.

457904-05: Porch, J. and H. Krueger. 2001. PS 149B1 Binary Insecticidal Crystal Protein: Dietary Toxicity to Parasitic Hymenoptera (*Nasonia vitripennis*): Final Report: Lab Project Number: 379-115. Unpublished study prepared by Wildlife International, Ltd. 27 p. {OPPTS 885.4340}

457904-06: Teixeira, D. 2001. Assessment of Chronic Toxicity of Diet Containing *Bacillus thuringiensis* PS149B1 Insecticidal Crystal Protein To Collembola (*Folsomia candida*): Lab Project Number: 12550.6142: 011106. Unpublished study prepared by Springborn Laboratories, Inc. 35 p.

457904-07: Sindermann, A., J. Porch, and H. Krueger. 2001. PS 149B1 Insecticidal Crystal Protein: Dietary Toxicity to Green Lacewing Larvae (*Chrysoperla carnea*): Final Report: Lab Project Number: 379-116A: 011104. Unpublished study prepared by Wildlife International, Ltd. 30 p. {OPPTS 885.4340}

457916-01: Bhatti, M., J. Duan, C. Pilcher, *et al*. 2002. Ecological Assessment for Non-target Organisms in the Plots of Corn Rootworm Insect Protected Corn Hybrid Containing MON 863 Event: 2000--2001 Field Trials: Lab Project Number: 00-01-39-16: MSL-17531. Unpublished study prepared by Monsanto Agronomy Center, AGVISE Laboratories, Monsanto Company and Key Agricultural Services, Inc. 143 p.

458086-01: Herman, R. 2002. Summary of Heat Lability Studies with Cry34Ab1/Cry35Ab1: Lab Project Number: GH-C 5603. Unpublished study prepared by Dow AgroSciences LLC. 21 p.

458602-01: Hunst, P. and R. Herman. 2002. Slide Presentation Summarizing Cry34Ab1/Cry35Ab1 Heat Inactivation Studies: Lab Project Number: DN0006331. Unpublished study prepared by Dow AgroSciences LLC. 18 p.

461239-05: Herman, R. 2003. Biological Equivalency of Cry35Ab1 Insecticidal Crystal Protein in Transgenic Plants and Derived from Transgenic *Pseudomonas fluorescens*. Project Number: GH/C/5696. Unpublished study prepared by Dow Agrosciences, LLC. 17 p.

461239-06: Schafer, B. 2003. Characterization of Cry34Ab1 and Cry35Ab1 Proteins Derived from Transgenic Maize Event E4497.59.1.22 (DAS-59122-7). Project Number: 030033. Unpublished study prepared by Dow AgroSciences LLC. 50 p.

461239-11: Smith, B., J. McNaughton, and M. Hinds. 2003. Nutritional Equivalency Study of Maize Containing Cry34Ab1 and Cry35Ab1: Poultry Feeding Study. Project Number: 2001/OPT/48/BB, PHI/2001/043. Unpublished study prepared by Woodson-Tenent Lab., Inc., Exygen Research and Pioneer Hi-Bred International, Inc. 33 p.

461239-12: Higgins, L. 2003. The Effect of Cry34Ab1/Cry35Ab1 Proteins on the Development and Mortality of the Ladybird Beetle *Coleomegilla maculata* DeGeer. Project Number: PHI/2003/045. Unpublished study prepared by Pioneer Hi-Bred International, Inc. 97 p.

461239-14: Higgins, L. and D. Wright. 2003. Evaluation of the Impact of Corn Rootworm Control Strategies on Non-Target Arthropods. Project Number: PHI/2001/020. Unpublished study prepared by Pioneer Hi-Bred International, Inc. 85 p.

465847-01: Cressman, R. 2003. Evaluation of the Sequence Similarities of the Cry34Ab1, Cry35Ab1, and PAT Proteins to the Public Protein Sequence Datasets. Project Number: PHI/2003/046. Unpublished study prepared by E.I. du Pont de Nemours and Co., Inc. 211 p.

Part 3.

Documents to facilitate harmonised safety assessments

Section 1.
OECD guidance for the designation of a unique identifier for transgenic plants

Foreword to the 2006 revised version

This guidance for a unique identifier for transgenic plants was developed by OECD's Working Group on Harmonisation of Regulatory Oversight in Biotechnology. The purpose is for use as a "key" to unlock or access information in OECD's database of products of modern biotechnology which have been approved for commercial application, as well as interoperable systems.

One of the first major steps in the development of this guidance was an OECD Workshop on Unique Identification Systems for Transgenic Plants, which was hosted by Switzerland in Charmey in October 2000. At the time of the Charmey Workshop, a number of options for developing a unique identifier were under consideration. Subsequently, these options (and related issues) were discussed in detail at the following meetings of the Working Group. At the 11th meeting of the Working Group (January 2002), delegations agreed on a guidance document that included an introductory section, a section on how to develop and generate unique identifiers, as well as a section on future developments. OECD's Business and Industry Advisory Committee (BIAC) have played an important part at all stages in the discussion through their Expert Group on Biotechnology. This is important because according to the guidance, it is the developers of transgenic products who will generate the unique identifier.

Since the first publication of this guidance document in 2002, the OECD's unique identification system for transgenic plants has been utilised without any major problems as "keys" to access information of each transgenic product in the Product Database (http://www.oecd.org/biotrack/productdatabase). In addition, it has been recognised as an appropriate identification system of products included in the Biosafety Clearing-house of the Cartagena Protocol on Biosafety developed under the auspices of the Convention on Biological Diversity (CBD).

With the recent increases of commercialisation of plant products having one or more traits obtained through the use of recombinant DNA techniques and stacked by conventional crosses in the backdrop, it was proposed to standardise the way to designate a unique identifier for such plant products. Up to that time, this guidance document had allowed two different options for such product in item 8.

At the 18th meeting of the Working Group (June 2006), the text modiying item 8 was agreed and the revised document could start its declassification process.

1. Introduction

The purpose of the unique identifier is for its use as a key to accessing information in the OECD product database and interoperable systems for the products of modern biotechnology which have been approved for commercial application. This guidance addresses the development of a unique identifier for use in the product database. It was developed from plant products in the OECD BioTrack Product Database and its use is directly applicable to plant products entered into

the database. While the concepts and principal components were developed for plants they may be considered for their potential applicability to other products.

OECD has been working on a "unique identifier for transgenic plants" since 2000. This was initiated with an OECD Workshop on Unique Identification Systems for Transgenic Plants, which was hosted by Switzerland in October of that year (Charmey, Switzerland, 2-4 October 2000).

A major objective was to identify the most efficient means of establishing a unique identifier for transgenic plants, and to draft conclusions, recommendations and points to consider within the context of OECD's Product Database. In this context, the Workshop proposed several options for a unique identifier. (See the "Report of the OECD Workshop on Unique Identification Systems for Transgenic Plants" http://www.oecd.org/biotrack)

There was a consensus that there is a need for a unique identifier: a simple alphanumeric code based on the transformation event (rather than other options such as a new variety), with a single digit for verification. The unique identifier should be a "key" to unlocking more detailed information in the product database and interoperable systems (for example, the Biosafety Clearing-House). As such, it should be kept short, simple and user friendly. It should also be built in a flexible way and might potentially serve as a core unique identifier for future developments. It should also take into account experience with, and be applicable to, existing products.

Each applicant has their own internal mechanism to avoid applying the same designation of the "transformation event" to different products. Consequently, incorporating the applicant information into the unique identifier is the only way to enable applicants to generate the unique identifier for their own product, while at the same time ensuring its uniqueness from those generated by other applicants. Furthermore, this provides applicants with the flexibility to generate the unique identifier at the time they believe appropriate or necessary.

2. Development and designation of the unique identifier

Item 1

The purpose of the unique identifier is for its use as a key to accessing information in the OECD product database and interoperable systems for the products of modern biotechnology which have been approved for commercial application. This guidance addresses the development of a unique identifier for use in the product database. It was developed from plant products in the OECD BioTrack Product Database and its use is directly applicable to plant products entered into the database. While the concepts and principal components were developed for plants they may be considered for their potential applicability to other products.

Item 2

Applicants should designate to the national authority a unique identifier for their product, at the latest, at the time of application for the first commercial approval.

Item 3

The national authority should, at the time of the first approval for commercialisation, notify the OECD BioTrack Product Database of the designated unique identifier, in order to enable access to the relevant information in the database for all subsequent applications for commercialisation in other countries.

Item 4

The unique identifier is a code of a fixed length of 9 alphanumeric digits for a transformation event derived from modern biotechnology.[12] It should be unique to that transformation event.

Item 5

The unique identifier is composed of three elements that must be separated by dashes (-). The total length is 9 digits, the last of which is a verification digit. The transformation event and the applicant designation should total 8 alphanumeric digits.

- 2 or 3 alphanumerical digits to designate the applicant;
- 5 or 6 alphanumerical digits to designate the "transformation event"[13];
- One numerical digit as a verification, as foreseen in item 7.

For example,

C	E	D	–	A	B	8	9	1	–	6

or

C	E	–	A	B	C	8	9	1	–	5

Item 6

The unique identifier should include the "applicant information" of 2 or 3 alphanumeric digits (for example, the first 2 or 3 digits of the applicant organisation name), followed by a dash. Any new applicant that is not identified within the database shall not be permitted to use the existing codes listed in the applicant's code table within the database. The applicant shall inform the national authorities who will update the BioTrack Product Database, by including a new code that will be designed to identify the new applicant in the code table.

Item 7

The unique identifier should include one verification digit, which shall be separated from the rest of the unique identifier digits by a dash. The verification digit is intended to reduce errors by ensuring the integrity of the alphanumeric code, entered by the users of the database.

The rule to calculate the verification digit is as follows. The verification digit is made up of a single numerical digit. It is calculated by adding together the numerical values of each of the alphanumerical digits in the unique identifier. The numerical value of each of the digits is from ∅ to 9 for the numerical digits (∅ to 9) and 1 to 26 for the alphabetical digits (A to Z) (see annex). The total sum, if made up of several numerical digits, will be further calculated by adding the remaining digits together using the same rule, in an iterative process, until the final sum is a single numerical digit.

---

[12] Zero should be reflected by the symbol ∅ to avoid confusion with the letter O.

[13] When the transformation event of an existing plant product, prior to the adoption of this guidance, is shorter or longer than 5 or 6 digits, the applicant should select 5 or 6 digits within the transformation event in order to fit it into this limit.

For example, the verification digit for the code CED-AB891 is calculated as follows:

- Step one : 3+5+4+1+2+8+9+1 = 33;
- Step two: 3+3 = 6; therefore the verification digit is 6;
- Therefore, this unique identifier then becomes CED-AB891-6

Item 8

The above guidance is sufficient to generate unique identifiers for the majority of existing plant products. Regarding plant products having two or more traits obtained through the use of recombinant DNA techniques and stacked by conventional crosses, the unique identifier should consist of the unique identifiers from each parental transgenic plant (*e.g.,* MON-15985-7 x MON-∅1445-2).

3. Future development

It was recognised that it may be necessary to revisit in the future the potential use of prefixes or suffixes if there is a need to incorporate further information fields. The use of prefixes or suffixes, on an *ad hoc* or voluntary basis, to incorporate further information fields for use in the BioTrack Product Database, as appropriate or requested by a country, will continue to be discussed and should be made public by national authorities.

This guidance for the development and designation of the unique identifier may be reassessed in the light of experience gained.

Annex

Form of digits to be used in the unique identifier

∅
1
2
3
4
5
6
7
8
9

Form of alphabetic characters to be used, plus numerical equivalents, for calculating verification digit

A=1
B=2
C=3
D=4
E=5
F=6
G=7
H=8
I=9
J=1∅
K=11
L=12
M=13
N=14
O=15
P=16
Q=17
R=18
S=19
T=2∅
U=21
V=22
W=23
X=24
Y=25
Z=26

Section 2
Molecular characterisation of plants derived from modern biotechnology

Foreword to the molecular characterisation document

The Working Group on the Harmonisation of Regulatory Oversight in Biotechnology and the Task Force for the Safety of Novel Foods and Feeds are implementing closely-related programmes of work at the OECD. Both of them develop science-based ***consensus documents***, which are mutually acceptable among member countries. These consensus documents contain information for use during the regulatory assessment of products derived from modern biotechnology.

In the area of plant biosafety (dealt with by the Working Group), consensus documents are being published on information on the biology of certain plant and animal species, selected traits that may be introduced into plant species, and environmental safety issues arising from certain general types of modifications made to crops, trees or micro-organisms.

In the area of food and feed safety (dealt with by the Task Force), consensus documents are focused on the nutrients, anti-nutrients or toxicants, the use as a food/feed and other relevant information on particular products. Reference is made to the concept of substantial equivalence, as it is considered that a comparative approach focusing on the determination of similarities and differences between the genetically engineered food and its conventional counterpart aids in the identification of potential safety and nutritional assessment.

The present *Consensus Document on Molecular Characterisation of Plants Derived from Modern Biotechnology* constitutes the first result from a joint collaborative project implemented from 2003 to 2010 by the Working Group and the Task Force. It addresses the issues linked to molecular characterisation in a risk/safety assessment. It describes the background and purpose of molecular characterisation, transformation methods, inserted DNA, insertion site and expressed material, inheritance and genetic stability. A summary is provided under section 5 of the document, and section 1.3 explains the scope of the text as follows:

"*The purpose of molecular characterisation is to inform the risk/safety assessment of plants derived from modern biotechnology. Such characterisation provides knowledge at the molecular level of the inserted DNA within the plant genome*[1]*, the insertion site and the expressed material (ribonucleic acid [RNA] and proteins), and may provide information on intended and possible unintended effects of the transformation. Molecular characterisation of the genotype*[2] *contributes to a rigorous assessment of the potential impacts of transformation on the food, feed and environmental risk/safety of a recombinant-DNA plant. It assists in the prediction of the phenotype and the phenotype will ultimately determine whether the recombinant-DNA plant poses any risk/safety concerns.*"

[1] Genome includes genetic material from both the nucleus and organelles.

[2] Genotype is defined as the genetic constitution of an organism.

1. Background

1.1. Molecular characterisation and risk/safety assessment

Molecular characterisation is one component of the science-based multi-disciplinary approach used in food, feed and environmental risk/safety assessment of plants derived from modern biotechnology. The molecular characterisation of these plants is used to gain an understanding of the genetic material introduced and expressed in them. The purpose of this document is to explain the scientific basis underlying the application of molecular characterisation to the food, feed and environmental risk/safety assessment of these plants.

This document is meant to inform a risk/safety assessor on the use of molecular characterisation data and information, which is one component of an overall risk/safety assessment. The document does not discuss which data and information should be considered by the competent authority conducting the risk/safety assessment because the use of the data and information considered may depend on the type of risk/safety assessment being performed as well as characteristics of the product. The document does not provide an exhaustive list of analytical techniques that may be used for molecular characterisation. Where examples of analytical techniques are given, these serve only to provide a better context for an aspect of molecular characterisation discussed and do not imply that specific techniques are recommended or necessary.

Modern biotechnology has been defined as "the application of a) *In vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or b) Fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection," in the Cartagena Protocol on Biosafety (SCBD, 2000) and by the Codex Alimentarius Commission (Codex, 2003a).

Notwithstanding the fact that plant varieties produced through all techniques, including conventional breeding methods, can pose risks, the scope of this document will be limited to plants produced using recombinant-DNA (rDNA) techniques and direct injection of nucleic acid into cells or organelles, referred to herein as recombinant-DNA plants[3]. More specifically, this document will examine the transformation process and vectors used during transformation; the genetic material delivered to the recipient plant; and the identification, inheritance and expression of the genetic material in the recombinant-DNA plant.

This document focuses on the subset of recombinant-DNA plants intended for commercialisation, unconfined or full release that is subject to risk/safety assessments.

For context, this subset of recombinant-DNA plants, subject to regulatory evaluation, has typically passed through a post-transformation screening and selection process. The development of new recombinant-DNA plants begins with the production of a large number of transformants (Padgette *et al.*, 1995; Zhou *et al.*, 2003; Heck *et al.*, 2005). Plants derived from the initial transformants are cultivated over several propagation cycles in order to identify those plants that stably express and inherit the intended phenotype[4] while maintaining desirable agronomic characteristics such as

[3] Other terms such as genetically modified plants, genetically engineered plants, transgenic plants and transformed plants are often used interchangeably with the term recombinant-DNA plant. For the purposes of this document, the term recombinant-DNA plant will be used specifically as defined in paragraph 4.

[4] Phenotype is defined as an observable characteristic or trait of an organism that is determined by interactions between its genotype and the environment, and may include but is not limited to physical, morphological, physiological and biochemical properties.

growth characteristics, fertility and yield. This screening and selection process helps developers identify plants exhibiting pleiotropic effects resulting from the transformation process. With each successive propagation cycle, crop developers discontinue development of plants that have unexpected or undesired traits. This process results in the selection of recombinant-DNA plants intended for commercialisation, unconfined or full release; the risk/safety assessment is performed on these recombinant-DNA plants.

1.2. National and international experience

Many national authorities with a history of regulating products of biotechnology have put in place standards and procedures for the pre-market assessment of recombinant-DNA plants and the products derived from them. The expertise and experience developed at the national level have been shared in a number of intergovernmental forums such as the Organisation for Economic Co-operation and Development (OECD), the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). The scientific principles and approach to risk/safety assessment, developed through consultation at the international level, are currently applied by regulatory agencies around the world. This document complements existing guidance developed by national authorities and international organisations in this area.

In the context of environmental risk/safety, several guidance documents have been developed that focus on an approach to evaluating environmental risk/safety, such as the *Safety Considerations for Biotechnology: Scale-up of Crop Plants* published by the OECD (1993). In addition, many other OECD documents, developed through consensus of the member countries, have provided the basis for environmental risk/safety assessment of recombinant-DNA plants.

In the context of food risk/safety, the Codex Alimentarius Commission, under the Joint FAO/WHO Food Standards Programme, has adopted several documents developed by the Codex *Ad Hoc* Intergovernmental Task Force on Foods Derived from Biotechnology, including the *Principles for the Risk Analysis of Foods Derived from Modern Biotechnology* (Codex, 2003a) and the *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants* (Codex, 2003b). In the context of feed risk/safety, the OECD has published *Considerations for the Safety Assessment of Animal Feedstuffs Derived from Genetically Modified Plants* (OECD, 2003). In addition, many other OECD documents, developed through consensus of the member countries, have provided the basis for food and feed risk/safety assessment of recombinant-DNA plants.

1.3. The purpose of molecular characterisation

The purpose of molecular characterisation is to inform the risk/safety assessment of plants derived from modern biotechnology. Such characterisation provides knowledge at the molecular level of the inserted DNA within the plant genome[5], the insertion site and the expressed material (ribonucleic acid [RNA] and proteins), and may provide information on intended and possible unintended effects of the transformation. Molecular characterisation of the genotype[6] contributes to a rigorous assessment of the potential impacts of transformation on the food, feed and environmental risk/safety of a recombinant-DNA plant. It assists in the prediction of the phenotype and the phenotype will ultimately determine whether the recombinant-DNA plant poses any risk/safety concerns.

[5] Genome includes genetic material from both the nucleus and organelles.

[6] Genotype is defined as the genetic constitution of an organism.

As it is generally considered by regulatory authorities, and in international consensus-building exercises, molecular characterisation encompasses a number of discrete considerations, including:

- The transformation method

  A description of the transformation method, together with a detailed description of any DNA sequences that could be potentially inserted into the plant genome;

- The inserted DNA, the insertion site and expressed material

  A description of the inserted DNA, including any genetic rearrangements, deletions or truncations that may have occurred as a consequence of the transformation, and the RNA and/or proteins expressed from the inserted DNA in various plant tissues and/or at different times during plant development; and

- Inheritance and genetic stability

  This addresses not only inheritance of the inserted DNA but also stability (*e.g.* translation or transcription) over multiple propagation cycles.

Molecular characterisation of the inserted DNA may be relevant in predicting possible unintended effects relevant to risk/safety, but it is not typically the primary means to detect such unintended effects. Other components of the risk/safety assessment including allergenicity and toxicological assessment of new substances (*e.g.* proteins, metabolites), changes in the levels of nutrients and anti-nutrients and of endogenous toxicants and allergens, or changes in plant fitness are integral for detecting unintended effects relevant to risk/safety.

Molecular characterisation for food, feed and environmental risk/safety assessment of recombinant-DNA plants is based on methods that target specific sequences and expressed products. New profiling technologies can provide information on many components at a particular level of biochemical/molecular organisation (*e.g.* transcriptomics – RNA; proteomics – proteins). While many of these new profiling technologies are under development, they are not as yet applied by national authorities in risk/safety assessment of recombinant-DNA plants. However, such technologies may serve as supplementary tools in risk/safety assessment in the future, provided they are sufficiently developed and validated. The potential applications of profiling technologies in the risk/safety assessment as well as the challenges associated with such applications have been discussed in several reviews (*e.g.* Kuiper *et al.*, 2003; Chassy *et al*., 2004) and are not addressed further in this document.

For context, unintended effects could arise from any form of plant breeding. For recombinant-DNA plants, these unintended effects may be due to the disruption of genomic sequences by the insertions, the action of transformation-induced genomic deletions and rearrangements, including within the inserted DNA, or pleiotropic effects caused by the new trait. Unintended effects may result in off-types that would be eliminated during the post-transformation screening and selection process. While both recombinant-DNA plants and conventionally bred plants, including those generated using techniques of mutagenesis, may be evaluated and selected for agronomic and morphological traits, typically most conventionally bred plants do not undergo a risk/safety assessment comparable to that performed for recombinant-DNA plants.

In conclusion, molecular characterisation is considered an important part of risk/safety assessment; however it is only one component in the overall approach to risk/safety assessment. Molecular characterisation complements other components of the risk/safety assessment, such as environmental, chemical, nutritional, allergenicity and toxicological data to compare the recombinant-DNA plant with its appropriate comparator. Of interest for the risk/safety assessment is whether plant transformation could inadvertently increase the potential toxicity or allergenicity of the recipient plant,

alter its nutritional quality, have negative environmental impacts or confer other undesirable traits. The totality of the available information relevant to risk/safety enables regulatory authorities to determine if a recombinant-DNA plant meets appropriate risk/safety standards.

2. Transformation methods

2.1. Introduction

Transformation is the process of inserting DNA sequences of interest into a plant genome. Different transformation methods are available and each method has associated characteristics that could influence the inserted DNA sequences that are integrated into the plant genome. For instance, the integration process could lead to rearrangements, deletions or multi-copy insertions as well as the insertion of 'other' sequences originating from either plasmid (vector) or chromosomal DNA. The presence of these 'other' DNA sequences is relevant to risk/safety assessment in so far as such sequences may result in the presence of new substances in the recombinant-DNA plant and may also lead to altered levels of RNAs and proteins. In this section, focus is put on DNA integration that might occur as a result of the particular transformation method employed.

Various methods are available for introducing DNA into the plant genome (reviewed by Hansen and Wright, 1999). The most commonly used bacterial-mediated plant transformation methods employ disarmed *Agrobacterium* spp. Other plant-associated bacteria outside the *Agrobacterium* genus might become important in plant transformation (Broothaerts *et al.*, 2005). Direct transformation methods include particle bombardment (also termed biolistics) and electroporation. Alternative methods (*e.g.* microinjection, electrophoresis) have been specifically designed for recalcitrant plant species or specific target tissues (Hansen and Chilton, 1996; reviewed by Rakoczy-Trojanowska, 2002). This section will focus on the most widely practiced transformation methods.

2.2. Agrobacterium-mediated transformation

During *Agrobacterium*-mediated transformation, a DNA region, termed T-DNA, flanked by short specific DNA stretches (*i.e.* T-DNA borders), is transferred and integrated in the plant genome (for review see Gelvin, 2003). Besides the T-DNA border sequences, virulence (*vir*) genes play a key role in the processing, export and integration of the T-DNA from the bacterium to the plant. In addition to their naturally *cis*-acting function, Vir proteins have been shown to be able to act in *trans*. Based on the latter finding, the so-called binary vector system, comprising i) a plasmid containing the DNA construct[7] flanked by T-DNA border sequences, and ii) a disarmed helper plasmid delivering the *vir* gene functions, has been developed. In order to disarm helper plasmids, T-DNA regions are removed. The binary vector system is nowadays most frequently applied in *Agrobacterium*-mediated transformation (Hellens *et al.*, 2000).

The *Agrobacterium* strain and helper plasmid used can be identified, and if previously uncharacterised a description can be provided. Information can also be provided on how the helper plasmid used was disarmed. In addition, the plasmid containing the DNA construct can be described. This information will reveal DNA sequences potentially transferred.

Agrobacterium-mediated transformation of plant tissue usually results in a low copy number of the DNA construct at a single insertion site. In some recombinant-DNA plant varieties reaching commercialisation T-DNAs have been found to be inserted as tandem repeats (direct or inverted in structure) at a single locus (reviewed by Smith *et al.*, 2001). Integration of incomplete T-DNA

[7] For the purposes of this document the term 'DNA construct' refers to the DNA intended for insertion into the plant genome.

sequences is also occasionally seen. Integration may be accompanied by several types of rearrangements of the DNA construct (duplications, inversions and interspersion with plant DNA) and of plant genomic DNA at the insertion site (duplications, inversions and translocations). The insertion of plasmid backbone sequences from outside the T-DNA borders is also sometimes observed (reviewed by Smith *et al.*, 2001), either with the right or the left T-DNA border sequences or as an independent unit unlinked from the T-DNA (Kononov *et al.*, 1997). Further consideration of the risk/safety assessment of these phenomena is given in Section III.

2.3. Direct transformation

Direct transformation of plant cells involves introducing the DNA sequences of interest directly to plant cells with the use of various techniques (*e.g.* particle bombardment, electroporation) that allow transport of the exogenous material across the cell wall and cell membrane. There is a possibility of introducing other DNA sequences not intended for transfer such as bacterial chromosomal DNA, depending on the purity of the DNA used for transformation. A description of the vector DNA, its preparation and its purity can be provided to reveal DNA sequences potentially transferred.

Direct transformation can be used with plant species not amenable to *Agrobacterium*-mediated transformation to successfully introduce new traits (see Taylor and Fauquet, 2002). Single integrants may be obtained if minimal expression cassettes (promoter, open reading frame and terminator) are used (Fu *et al.*, 2000). Particle bombardment may lead to insertion of multiple copies of the DNA construct (in direct or inverted repeat structure) at a single or multiple loci (Jackson *et al.*, 2001; reviewed by Smith *et al.*, 2001). Multiple copies of the DNA construct at a single insertion site may have short stretches of plant genomic DNA interspersed between them. In some cases, the introduced DNA may have undergone deletions or rearrangements, such as concatamerisation (reviewed by Smith *et al.*, 2001). Vector backbone DNA might also be present in recombinant-DNA plants produced using whole plasmids or in cases where purified expression cassettes were used for transformation and the expression cassettes were not sufficiently purified.

2.4. Conclusions

A description of the transformation method employed provides information about the DNA sequences potentially transferred to the plant genome and can be valuable for identifying changes to the plant in order to focus subsequent aspects of the risk/safety assessment.

3. Inserted DNA, the insertion site and expressed material

3.1. Inserted DNA and insertion site

In a risk/safety assessment, the analysis of the inserted DNA can be used to characterise the genotype arising from the transformation. Data defining whether deletions and/or rearrangements have occurred in the DNA construct or at the insertion site can be used to identify whether there may be potential effects other than the intent of the original transformation. In this section, information on the inserted DNA and the changes at the insertion site resulting from the transformation are discussed.

It should be noted that in this section the analysis of the inserted DNA is considered to be part of an assessment where the inserted DNA is stably inherited in recombinant-DNA plants intended for commercialisation, unconfined or full release, as discussed in paragraph 6.

3.1.1. Integration and copy number

Insertion of a DNA construct can either occur in the nuclear plant genome or in the genome of organelles, such as chloroplasts. Information on whether an insertion is located in the nucleus or

an organelle can inform the environmental risk/safety assessment with regard to the potential dispersal of the gene of interest in relation to the reproductive biology of the recombinant-DNA plant. If the inserted DNA is located in the chloroplasts, it will most likely only be inherited maternally [most higher plants transmit their chloroplast DNA (predominantly) maternally rather than through pollen dispersal (Bock, 2007)]. Inserted DNA will be inherited both maternally and paternally when located in the nucleus. Molecular analysis and inheritance studies can provide information on the location of the inserted DNA (see also Section IV).

Depending on the transformation method used, the number of insertion sites might vary. In addition, there may be multiple copies of the DNA construct at each insertion site (see also Section II). Although plants with a single copy of the DNA construct are typically selected, in some cases plants with multiple copies of the DNA construct may be more efficacious as they result in higher expression levels. Copy number may influence gene silencing; however, copy number may not be as relevant as the homology of the introduced DNA to endogenous genes (Flavell, 1994).

Using appropriate controls, experimental data (*e.g.* Southern blot analysis) may reveal information such as the number of insertion sites, the copy number at each site and the genetic elements (*e.g.* promoters, enhancers) that have been inserted.

3.1.2. Presence of plasmid backbone sequences

Integration of DNA vector backbone sequence into the plant genome can occur with both *Agrobacterium*-mediated and direct transformation methods (see also Section II). Incorporation of DNA vector backbone sequences may be important if it results in the expression of additional proteins (for discussion see paragraph 33) or alters endogenous gene expression. Therefore, Southern blots of genomic DNA may be probed with DNA sequences from vector backbone(s) to determine if these elements have been inserted.

3.1.3. Organisation of transforming DNA and sites of insertion

The DNA used for transformation may be rearranged during the process of integration into the plant genome. Sequence analysis, polymerase chain reaction (PCR) analysis of the inserted DNA and Southern blotting are techniques that can be used to identify such rearrangements. If experimental results indicate a complex insert, such as one with rearrangements or deletions, further analysis may be useful to characterise the inserted DNA for the purposes of determining whether new substances may be present in the plant that could be relevant to the phenotype of the plant. These rearrangements may not necessarily be significant with regard to food, feed and/or environmental risk/safety.

T-DNA integration into an endogenous gene's coding or regulatory sequence and deletions or rearrangements of plant genomic DNA at the insertion site may cause loss of endogenous gene function or alteration of endogenous gene expression. This may result in changes in the plant which may or may not be significant with respect to risk/safety. Analysis of the regions flanking the inserted DNA may be used to determine if the DNA construct has been inserted in an endogenous gene's coding or regulatory sequence, and for the identification of any potential effects on plant gene function. The ability to analyse changes at the insertion site regarding the loss of plant gene function is, however, often compromised by lack of knowledge of most gene functions. Characterisation of insertion sites could inform the subsequent analyses for unintended effects that are part of the agronomic, phenotypic and compositional assessment of the plant (as discussed in paragraph 12).

New open reading frames (ORFs) might be formed as a result of transformation, potentially leading to the production of new proteins. DNA sequence analysis of the regions spanning the inserted DNA-genomic DNA junctions may reveal the presence of new ORFs as well as the presence of regulatory sequences upstream or downstream of the new ORF.

3.2. Expressed material

Expression of the inserted DNA is taken into account in order to evaluate the risk/safety of the new gene products on food, feed and the environment. Expression of vector backbone sequences and new ORFs may also be considered. Data obtained through molecular analysis should reveal whether the inserted vector DNA can be transcribed and translated. If potential new ORFs are identified, bioinformatics tools can assist to determine the likelihood of RNA formation, the possibility for transcription and translation to occur and the amino acid sequence of the putative new protein. If it is found that new proteins are likely produced, their potential impact on risk/safety should be fully characterised. The risk/safety assessment of any new protein is outside the scope of this document.

In some cases the intended goal of the insertion of the DNA construct is to suppress or down regulate the transcription of an endogenous target gene. In these cases, protein expression of the endogenous target gene will be reduced or inhibited. In some cases gene silencing constructs may also influence, as an unintended effect, the transcription or translation of other endogenous genes sharing significant sequence similarity.

3.2.1. Transcription and translation

Successful transfer of a DNA construct into a new plant variety does not necessarily mean the construct will be expressed (Gelvin, 2003). Several factors can influence the level and stability of expression of the inserted DNA. The copy number of the insert, the structure of the inserted DNA (*e.g.* presence of inverted repeats) and the insertion site have been shown to affect transcription (Flavell, 1994**;** Gelvin, 1998; Matzke and Matzke, 1998). Moreover, where and when the inserted DNA is actively transcribed depends, in part, on the promoters used (*e.g.* tissue-specific promoters may limit expression to desired tissues), the developmental stages (*e.g.* flowering, seed setting) of the plant and the environment in which the recombinant-DNA plant is grown (Bregitzer and Tonks, 2003; Zhu *et al.*, 2004).

Expression of the inserted DNA can be determined by use of either nucleic acid techniques such as northern blotting to detect recombinant RNA or by antibody-based methods such as western blotting to detect protein encoded by the inserted DNA. When performing analyses to characterise the expression of the inserted DNA, care should be taken to ensure that the conditions used for analysis (such as the tissues examined and the growth conditions used) are relevant to the risk/safety assessment. Once identified, the expression products from the inserted DNA can be characterised and assessed for risk/safety.

Expression of the inserted DNA in relevant tissues and under relevant environmental conditions is taken into consideration when assessing exposure and is considered as part of the subsequent risk/safety assessment. The stable integration in the plant genome does not imply that inserted DNA expression would, nor should, be expected to occur at steady state levels through the life cycle of the recombinant-DNA plant. Analysis of plant tissues at key developmental stages for proteins encoded by the inserted gene would reveal the amount of proteins produced at those developmental stages relevant to the risk/safety assessment, such as whether the protein is present in food and feed, or at which developmental phases environmental exposure will be most significant (*e.g.* expression of the protein in pollen).

3.2.2. Post-translational modification

Following translation, a protein can undergo further modifications. Identifying and characterising the proteins encoded by the inserted gene(s) can provide information useful in confirming that the substances expressed are those that the developer intended to express. Characterising these proteins can create a link to the history of safe use, where relevant, by showing that the proteins expressed *in planta* are not meaningfully different from the proteins when expressed in their native hosts. This is

necessary in order to ensure that the data and information about the proteins in their native hosts that may be referenced in the risk/safety assessment of the recombinant-DNA plant are relevant. Algorithms to identify potential post-translation modification such as N- and O-glycosylation sites, Ser/Thr/Tyr phosphorylation sites and (iso)prenylation have been developed (Blom *et al*., 2004; Maurer-Stroh and Eisenhaber, 2005). Protein analysis studies applying specific staining methods, radioactive labelling studies or matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) may demonstrate the presence of the predicted post-translational modifications (Jensen, 2000) that are deemed relevant to the risk/safety assessment. While some of these post-translational modifications might impact on the risk/safety of the protein, these considerations fall beyond the scope of molecular characterisation but should be considered as part of the overall risk/safety assessment.

3.3. Conclusions

The analysis of the inserted DNA can be useful in the characterisation of the genotype arising from the transformation. Deletions and/or rearrangements that may have occurred in the DNA construct or at the insertion site may result in effects other than the intent of the original transformation. Analysis of expressed products is important for the assessment of the phenotype; however, it must be considered in the context of a complete risk/safety assessment.

4. Inheritance and genetic stability

4.1. Introduction

Information regarding the inheritance and genetic stability of the inserted DNA is used to extend the conclusions of a risk/safety assessment conducted for a particular propagation cycle of the recombinant-DNA plant to subsequent genetic descendants. Therefore, information regarding the inheritance and genetic stability of the inserted DNA is important and necessary in the assessment of food, feed and environmental risk/safety.

Inheritance is defined as the pattern of transmission of genotype and phenotype into genetic descendants. The stability of a genetic modification is defined as maintenance of the integrity of the original structure and function of the modification over time and over propagation cycles. Genetic stability can be confirmed by conducting genotypic analysis at the insertion site and/or by phenotypic analysis for expression of the desired trait in the course of plant production and propagation.

4.2. Inheritance and genetic stability in risk/safety assessment

Genetic stability and inheritance of introduced traits within and across propagation cycles are considered as part of the risk/safety assessment. Analysis of inheritance includes consideration of whether the inserted DNA is located on a nuclear plant chromosome or in plant organelles and whether it is transferred into offspring maternally or paternally. Demonstrating that the inserted DNA has been stably integrated into the genome provides some assurance that a risk/safety assessment performed on an early propagation cycle of the plant is applicable to future propagation cycles of the plant. For context, when selecting plants for commercialisation, unconfined or full release, developers typically look for plants in which the inserted DNA has been stably integrated into the genome.

4.2.1. Patterns of inheritance

In the case of insertion of the DNA construct into the nucleus, predictable patterns of inheritance are typically reflected in Mendelian segregation ratios for phenotype and genotype. Deviations from Mendelian inheritance are potential indicators of genetic instability, especially for chromosomal genetic modifications of the nuclear genome in diploid, sexual plants that form the majority of new plants

typically encountered by regulators. However, the patterns of inheritance applicable to a particular plant species depend on the mechanisms of inheritance that exist for the subject plant species such as the reproductive strategy, the ploidy and whether nuclear or organelle genomes are involved.

Mendelian inheritance would not be expected for all asexual, vegetatively propagated plants, some polyploids and all genetic modifications of plastid or mitochondrial genomes. Such expected instances of non-Mendelian inheritance should not be interpreted as genetic instability.

4.2.2. Factors of genetic stability

As in all plants, genotypic change may occur over the course of mitotic or meiotic cell division and the transmission of genes into resulting progeny. Spontaneous mutations could occur due to errors in base pair incorporation during DNA replication and chromosome doubling prior to mitotic cell division. The pairing of homologous chromosomes during meiosis can lead to crossing over, a recombination that may result in a new grouping of genes. The stability of the inserted DNA may also depend on the sequence and structure of the introduced or modified genes and on characteristics of the insertion site.

4.2.3. Methods to determine the stability of a genetic modification

The stability of a genetic modification may be analysed at the phenotypic and/or the genotypic level. The stability of phenotypic expression may be determined by trait characterisation or by analysis of sufficient samples, where appropriate, of RNA or protein expression. Some phenotypic traits (*e.g.* resistances) may be quantified under testing conditions with the intact plant. As with other plant genes, expression of inserted DNA will be influenced by the environment. This should be taken into account during a phenotypic consideration of stability. Changes in patterns of expression or expression levels can be quantified in a biochemical reaction mediated by an expressed enzyme or by detection of the expressed protein with specific antibodies (*e.g.* enzyme-linked immunosorbent assay [ELISA], western blot analysis).

The stability of a genetic modification at the genotypic level may be documented through comparative analyses of the structure of the genetic modification using techniques such as Southern blot, PCR or other types of genetic analysis of multiple plants within and across propagation cycles. Genotypic changes across propagation cycles in the recombinant-DNA plant should be considered in the context of the normal variation that occurs with plant breeding.

4.3. Conclusions

Inheritance and genetic stability can inform the food, feed and environmental risk/safety assessment. This information is important in extending the conclusions of a risk/safety assessment conducted for particular propagation cycles of the recombinant-DNA plant to subsequent genetic descendants.

5. Summary

Molecular characterisation encompasses consideration of the transformation method employed, the inserted DNA and expressed material, and the inheritance and genetic stability of the inserted DNA. Molecular characterisation in and of itself is not a sufficient means of predicting the risk/safety of recombinant-DNA plants. However, molecular characterisation may be useful in focusing other components of the risk/safety assessment that assess the phenotype of the plant, such as characterisation of the levels of nutrients, anti-nutrients, endogenous toxicants or allergens, or changes in plant fitness. To date, the most appropriate available scientific procedures and technology have been used in the molecular characterisation of recombinant-DNA plants. Experience from the use of these procedures and technology form the basis of this document. Based on the current pace of technological advancement, it is expected that new methodologies may be applied to the molecular characterisation of recombinant-DNA plants should such technologies prove to have added value as a mechanism of hazard identification in food, feed and environmental risk/safety assessments.

References

Blom, N., T. Sicheritz-Pontén, R. Gupta, S. Gammeltoft and S. Brunak (2004), "Prediction of Post-Translational Glycosylation and Phosphorylation of Proteins from the Amino Acid Sequence", *Proteomics Vol. 4*: 1633-1649.

Bock, R. (2007), "Structure, Function, and Inheritance of Plastid Genomes", In R. Bock, ed., *Cell and Molecular Biology of Plastids. Topics in Current Genetics Vol. 19*, pp. 29-63, Springer-Verlag, Berlin, Germany.

Bregitzer, P. and D. Tonks (2003), "Inheritance and Expression of Transgenes in Barley", *Crop Science Vol. 43*, pp. 4-12.

Broothaerts, W., H.J. Mitchell, B. Weir, S. Kaines, L.M.A. Smith, W. Yang, J.E. Mayer, C. Roa-Rodriguez and R.A. Jefferson (2005), "Gene Transfer to Plants by Diverse Species of Bacteria", *Nature Vol. 433*, pp. 629-633.

Chassy, B., J.J. Hlywka, G.A. Kleter, E.J. Kok, H.A. Kuiper, M. McGloughlin, I.C. Munro, R.H. Phipps and J.E. Reid (2004), "Nutritional and Safety Assessments of Foods and Feeds Nutritionally Improved Through Biotechnology", *Comprehensive Reviews in Food Science and Food Safety Vol. 3*, pp. 35-104.

Codex Alimentarius Commission (Codex) (2003a, with amendment in 2008), *Principles for the Risk Analysis of Foods Derived from Modern Biotechnology*, CAC/GL 44-2003, available online at http://www.codexalimentarius.net/download/standards/10007/CXG_044e.pdf

Codex (2003b; with Annexes II and III adopted in 2008), *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants*, CAC/GL 45-2003, available online at http://www.codexalimentarius.net/download/standards/10021/CXG_045e.pdf

Flavell, R.B. (1994), "Inactivation of Gene Expression in Plants as a Consequence of Specific Sequence duplication", *Proceedings of the National Academy of Sciences of the United States of America Vol. 91*, pp. 3490-3496.

Fu, X., L.T. Duc, S. Fontana, B.B. Bong, P. Tinjuangjun, D. Sudhakar, R.M. Twyman, P. Christou and A. Kohli (2000), "Linear Transgene Constructs Lacking Vector Backbone Sequences Generate Low-Copy-Number Transgenic Plants with Simple Integration Patterns", *Transgenic Research Vol. 9*, pp. 11-19.

Gelvin, S.B. (1998), "The Introduction and Expression of Transgenes in Plants", *Current Opinion in Biotechnology Vol. 9*, pp. 227-232.

Gelvin, S.B. (2003), "*Agrobacterium*-mediated Plant Transformation: the Biology behind the 'Gene-jockeying' Tool", *Microbiology and Molecular Biology Reviews Vol. 67*, pp. 16-37.

Hansen, G. and M.D. Chilton (1996), "'Agrolistic' Transformation of Plant Cells: Integration of T-Strands Generated *in Planta*", *Proceedings of the National Academy of Sciences of the United States of America Vol. 93*, pp. 14978-14983.

Hansen, G. and M.S. Wright (1999), "Recent Advances in the Transformation of Plants", *Trends in Plant Science Vol. 4*, pp. 226-231.

Heck, G.R., C.L. Armstrong, J.D. Astwood, C.F. Behr, J.T. Bookout, S.M. Brown, T.A. Cavato, D.L. DeBoer, M.Y. Deng, C. George, J.R. Hillyard, C.M. Hironaka, A.R. Howe, E.H. Jakse, B.E. Ledesma, T.C. Lee, R.P. Lirette, M.L. Mangano, J.N. Mutz, Y. Qi, R.E. Rodriguez, S.R. Sidhu, A. Silvanovich, M.A. Stoecker, R.A. Yingling and J. You (2005), "Development and Characterization of a CP4 EPSPS-Based Glyphosate-Tolerant Corn Event", *Crop Science Vol. 44*, pp. 329-339.

Hellens, R., P. Mullineaux and H. Klee (2000), "A Guide to *Agrobacterium* Binary Ti Vectors", *Trends in Plant Science Vol. 5*, pp. 446-451.

Horvath, H., L.G. Jensen, O.T. Wong, E. Kohl, S.E. Ullrich, J. Cochran, C.G. Kannangara and D. von Wettstein (2001), "Stability of Transgene Expression, Field Performance and Recombination Breeding of Transformed Barley Lines", *Theoretical and Applied Genetics Vol. 102*, pp. 1-11.

Jackson, S.A, P. Zhang, W.P. Chen, R.L. Philips, B. Friebe, S. Muthukrishnan and B.S. Gill (2001), "High-Resolution Structural Analyis of Biolistic Transgene Integration into the Genome of Wheat", *Theoretical and Applied Genetics Vol. 103*, pp. 56-62.

Jensen, O.N. (2000), "Modification-Specific Proteomics: Systematic Strategies for Analysing Post-Translationally Modified Proteins", *Trends in Biotechnology Vol.18, Supplement 1*, pp. 36-42.

Kononov, M.E., B. Bassuner and S.B. Gelvin (1997), "Integration of T-DNA Binary Vector 'Backbone' Sequences into the Tobacco Genome: Evidence for Multiple Complex Patterns of Integration", *The Plant Journal Vol.11*, pp. 945-957.

Kuiper, H.A., E.J. Kok and K.-H. Engel (2003), "Exploitation of Molecular Profiling Techniques for GM Food Safety Assessment", *Current Opinion in Biotechnology Vol. 14*, pp. 238-243.

Matzke, A.J.M. and M.A. Matzke (1998), "Position Effects and Epigenetic Silencing of Plant Transgenes", *Current Opinion in Plant Biology Vol. 1*, pp. 142-148.

Maurer-Stroh, S. and F. Eisenhaber (2005), "Refinement and Prediction of Protein Prenylation Motifs", *Genome Biology Vol.6*, R55.

Muskens, M.W.M., A.P.A. Vissers, J.N.M. Mol and J.M. Kooter (2000), "Role of Inverted DNA Repeats in Transcriptional and Post-transcriptional Gene Silencing", *Plant Molecular Biology Vol. 43*, pp. 243-260.

OECD (Organisation for Economic Co-operation and Development) (1993), *Safety Considerations for Biotechnology: Scale-Up of Crop Plants*, OECD, Paris.

OECD (2003), "Considerations for the Safety Assessment of Animal Feedstuffs Derived from Genetically Modified Plants", *Series on the Safety of Novel Foods and Feeds No. 9*, OECD Environment Directorate, Paris.

Padgette, S.R., K.H. Kolacz, X. Delannay, D.B. Re, B.J. LaVallee, C.N. Tinius, W.K. Rhodes, Y.I. Otero, G.F. Barry, D.A. Eichholtz, V.M. Peschke, D.L. Nida, N.B. Taylor and G.M. Kishore (1995), "Development, Identification, and Characterization of a Glyphosate-Tolerant Soybean Line", *Crop Science Vol. 35*: 1451-1461.

Rakoczy-Trojanowska, M. (2002), "Alternative Methods of Plant Transformation – a Short Review", *Cellular & Molecular Biology Letters Vol. 7*, pp. 849-858.

SCBD (Secretariat of the Convention on Biological Diversity) (2000), *Cartagena Protocol on Biosafety to the Convention on Biological Diversity: Text and Annexes*, Secretariat of the Convention on Biological Diversity, Montreal, Canada.

Smith, N., J.B. Kilpatrick and G.C. Whitelam (2001), "Superfluous Transgene Integration in Plants", *Critical Reviews in Plant Sciences Vol. 20*, pp. 215-249.

Tang, J., R. Scarth and P.B.E. McVetty (2004), "Stability of the Expression of Acyl-ACP Thioesterase Transgenes in Oilseed Rape Doubled Haploid Lines", *Crop Science Vol. 44*, pp. 732-740.

Taylor, N.J. and C.M. Fauquet (2002), "Microparticle Bombardment as a Tool in Plant Science and Agricultural Biotechnology", *DNA and Cell Biology Vol. 21*, pp. 963-977.

Yin, Z., M. Szwacka, R. Malinowski and S. Malepszy (2004), "Differences in the Inheritance Stability of Kanamycin Resistance between Transgenic Cucumbers (*Cucumis sativus* L.) Containing two Constructs", *Journal of Applied Genetics Vol. 45*, pp. 307-313.

Zhang, Y., X. Yin, A. Yang, G. Li and J. Zhang (2005), "Stability of Inheritance of Transgenes in Maize (*Zea mays* L.) Lines Produced using Different Transformation Methods", *Euphytica Vol. 144*, pp. 11-22.

Zhou, H., J.D. Berg, S.E. Blank, C.A. Chay, G. Chen, S.R. Eskelsen, J.E. Fry, S. Hoi, T. Hu, P.J. Isakson, M.B. Lawton, S.G. Metz, C.B. Rempel, D.K. Ryerson, A.P. Sansone, A.L. Shook, R.J. Starke, J.M. Tichota and S.A. Valenti (2003), "Field Efficacy Assessment of Transgenic Roundup Ready Wheat", *Crop Science Vol. 43*, pp. 1072-1075.

Zhu, B., J.R. Lawrence, S.I. Warwick, P. Mason, L. Braun, M.D. Halfhill and C.N. Stewart Jr. (2004), "Stable *Bacillus thuringiensis* (Bt) Toxin Content in Interspecific F_1 and Backcross Populations of Wild *Brassica rapa* after Bt Gene Transfer", *Molecular Ecology Vol. 13*, pp. 237-241.

List of OECD consensus documents 1996-2010

	Consensus Document	Lead Country(ies)	Year of Issue	Volume
Biology of Crops	Oilseed Rape (*Brassica napus*)	Canada	1997	Vol. 1
	Potato (*Solanum tuberosum* subsp. *tuberosum*)	Netherlands & United Kingdom	1997	Vol. 1
	Wheat (*Triticum aestivum*)	Germany	1999	Vol. 1
	Rice (*Oryza sativa*)	Japan	1999	Vol. 1
	Soybean (*Glycine max*)	Canada	2000	Vol. 1
	Sugar Beet (*Beta vulgaris*)	Switzerland	2001	Vol. 1
	Maize (*Zea mays* subs. *mays*)	Mexico	2003	Vol. 1
	Sunflower (*Helianthus annus*)	France	2004	Vol. 1
	Papaya (*Carica papaya*)	United States	2005	Vol. 1
	Oyster Mushroom (*Pleurotus* spp.)	Korea	2005	Vol. 1
	Chili, Hot & Sweet Peppers (*Capsicum annuum*)	Korea, Mexico & United States	2006	Vol. 1
	Cotton (*Gossypium* spp.)	Spain	2008	Vol. 4
	Bananas & Plantains (*Musa* spp.)	Spain	2009	Vol. 4

Facilitating Harmonisation	Introduction to the OECD Biosafety Consensus Documents *(Available in English and French)*	Working Group	2005	Vol. 1, 3 & 4
	Points to Consider for Consensus Documents on Biology of Cultivated Plants	Working Group	2006	Vol. 3
	Designation of a Unique Identifier for Transgenic Plants (Revised Version) (Guidance Document)	Working Group	2006	Vol. 3
	Molecular Characterisation of Plants Derived from Modern Biotechnology	Canada	2010	Vol. 3

	Consensus Document	Lead Country(ies)	Year of Issue	Volume
Biology of Trees	*Trees*			
	Norway Spruce (*Picea abies*)	Norway	1999	Vol. 2
	White Spruce (*Picea glauca*)	Canada	1999	Vol. 2
	Poplars (*Populus* spp.)	Canada	2000	Vol. 2
	Sitka Spruce (*Picea sitchensis*)	Canada	2002	Vol. 2
	Eastern White Pine (*Pinus strobus*)	Canada	2002	Vol. 2
	European White Birch (*Betula pendula*)	Finland	2003	Vol. 2
	White Pine (*Pinus monticola*)	Canada	2008	Vol. 3
	Jack Pine (*Pinus banksiana*)	Canada	2006	Vol. 3
	North American Larches (*Larix lyalli, Larix occidentalis, Larix laricina*)	Canada	2007	Vol. 3
	Douglas-Fir (*Pseudotsuga menziesii*)	Canada	2008	Vol. 3
	Lodgepole Pine (*Pinus contorta*)	Canada	2008	Vol. 3
	Black Spruce (*Picea mariana*)	Canada	2010	Vol. 3
	Fruit Trees			
	Stone Fruits (*Prunus* spp.)	Austria	2002	Vol. 2
	Papaya (*Carica papaya*) [listed above in "Crops"]	United States	2005	Vol. 1
	Bananas & Plantains (*Musa* spp.) [listed above in "Crops"]	Spain	2009	Vol. 4

	Consensus Document	Lead Country(ies)	Year of Issue	Volume
Traits	Crop Plants Made Virus Resistant through Coat Protein Gene-Mediated Protection	Task Group	1996	Vol. 1
	Genes and their Enzymes that Confer Tolerance to Glyphosate Herbicide	United States, Germany & Netherlands	1999	Vol. 1
	Genes and their Enzymes that Confer Tolerance to Phosphinothricin Herbicide	United States, Germany & Netherlands	1999	Vol. 1
	Herbicide Metabolism and the Residues in Glufosinate-Ammonium (Phosphinothricin)-Tolerant Transgenic Plants	Germany	2002	Vol. 1
	Transgenic Plants Expressing *Bacillus thuringiensis*-Derived Insect Control Protein	United States	2007	Vol. 3
Micro-organisms	*Info. used in the Assessment of Env. Applications of Micro-organisms*			
	Pseudomonas	United Kingdom	1997	Vol. 2
	Acidithiobacillus	Canada	2006	Vol. 2
	Baculovirus	Germany	2002	Vol. 2
	Acinetobacter	Canada	2008	Vol. 4
	Guidance Documents on Biosafety Aspects of Bacteria			
	Use of Taxonomy in Risk Assessment of Micro-organisms: Bacteria	Canada & United States	2003	Vol. 4
	Methods for Detection of Micro-organisms Introduced into the Environment: Bacteria	Netherlands	2004	Vol. 4
	Horizontal Gene Transfer Between Bacteria	Germany	2010	Vol. 4

List of OECD consensus documents by volume

Volume 1

- Biology of crops
    - Oilseed Rape (*Brassica napus*)
    - Potato (*Solanum tuberosum* subs. *Tuberosum*)
    - Wheat (*Triticum aestivum*)
    - Rice (*Oryza sativa*)
    - Soybean (*Glycine max*)
    - Sugar Beet (*Beta vulgaris*)
    - Maize (*Zea mays* subs. *mays*)
    - Sunflower (*Helianthus annus*)
    - Papaya (*Carica papaya*)
    - Oyster Mushroom (*Pleurotus* spp.)
    - Chili, Hot & Sweet Peppers (*Capsicum annuum*)
- Traits
    - Crop plants made virus resistant trough coat protein gene-mediated protection
    - Genes and their enzymes that confer tolerance to glyphosate herbicide
    - Genes and their enzymes that confer tolerance to phosphinothricin herbicide
    - Herbicide metabolism and the residues in glufosinate-ammonium (phosphinothricin) - tolerant transgenic plants

Volume 2

- Biology of trees
    - Norway spruce (*Picea abies*)
    - White spruce (*Picea glauca*)
    - Sitka spruce (*Picea sitchensis*)
    - Eastern white pine (*Pinus strobus*)
    - Poplars (*Populus* spp.)
    - Stone fruits (*Prunus* spp.)
    - European white birch (*Betula pendula*)
- Micro-organims
    - *Pseudomonas*
    - *Acidithiobacillus*
    - Baculovirus

Volume 3

- Biology of trees
    - White pine (*Pinus monticola*)
    - Jack pine (*Pinus banksiana*)
    - North american larches (*Larix lyalli*, *Larix occidentalis*, *Larix laricina*)
    - Douglas-Fir (*Pseudotsuga menziesii*
    - Lodgepole pine (*Pinus contorta*)
    - Black spruce (*Picea mariana*)
- Traits
    - Safety information on transgenic plants expressing *Bacillus thuringiensis*–Derived insect control protein
- Facilitating harmonisation
    - Guidance for designation of a unique identifier for transgenic plants
    - Molecular characterization of plants derived from modern biotechnology

Volume 4

- Biology of crops
    - Cotton (*Gossypium* spp.)
    - Bananas and plantains (*Musa* spp.)
- Micro-organisms
    - *Acinetobacter*
    - Use of taxonomy in risk assessment of micro-organisms: Bacteria
    - Methods for detection of micro-organisms intoduced into the environmental: Bacteria
    - Horizontal gene transfer between bacteria

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

The OECD is a unique forum where governments work together to address the economic, social and environmental challenges of globalisation. The OECD is also at the forefront of efforts to understand and to help governments respond to new developments and concerns, such as corporate governance, the information economy and the challenges of an ageing population. The Organisation provides a setting where governments can compare policy experiences, seek answers to common problems, identify good practice and work to co-ordinate domestic and international policies.

The OECD member countries are: Australia, Austria, Belgium, Canada, Chile, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Korea, Luxembourg, Mexico, the Netherlands, New Zealand, Norway, Poland, Portugal, the Slovak Republic, Slovenia, Spain, Sweden, Switzerland, Turkey, the United Kingdom and the United States. The European Commission takes part in the work of the OECD.

OECD Publishing disseminates widely the results of the Organisation's statistics gathering and research on economic, social and environmental issues, as well as the conventions, guidelines and standards agreed by its members.

OECD PUBLISHING, 2, rue André-Pascal, 75775 PARIS CEDEX 16
(97 2010 16 1 P) ISBN 978-92-64-09542-7 – No. 57737 2010

Safety Assessment of Transgenic Organisms

OECD CONSENSUS DOCUMENTS

Volume 3

The books on "Safety Assessment of Transgenic Organisms" constitute a compilation of the OECD Biosafety Consensus Documents. When published, Volume 1 and 2 contained the documents issued before 2006; Volume 3 and 4 are a continuation of the compilation up to 2010.

The OECD Biosafety Consensus Documents identify elements of scientific information used in the environmental safety and risk assessment of transgenic organisms which are common to OECD member countries and some non members associated with the work. This is intended to encourage information sharing, promote harmonised practices, and prevent duplication of effort among countries.

These books offer ready access to those consensus documents which have been issued on the website thus far. As such, it should be of value to applicants for commercial uses of transgenic organisms (crops, trees, micro-organisms), to regulators and risk assessors in national authorities, as well as the wider scientific community.

More information on the OECD's work related to the biosafety of transgenic organisms is found at BioTrack Online (***http://www.oecd.org/biotrack***).

Further reading

Safety Assessment of Transgenic Organisms – OECD Consensus Documents, Vol 1 (2006)
Safety Assessment of Transgenic Organisms – OECD Consensus Documents, Vol 2 (2006)

Related reading

The Bioeconomy to 2030: Designing a Policy Agenda (2009)
La bioéconomie à l'horizon 2030: quel programme d'action ? (2009)
OECD Biotechnology Statistics 2009 (2009)
Cutting Costs in Chemicals Management: How OECD Helps Governments and Industry (2010)
Réduire les coûts de gestion des produits chimiques : Comment l'OCDE aide les gouvernements et l'industrie (2010)

Please cite this publication as:

OECD (2010), *Safety Assessment of Transgenic Organisms: OECD Consensus Documents: Volume 3*, OECD Publishing.
http://dx.doi.org/10.1787/9789264095434-en

This work is published on the *OECD iLibrary,* which gathers all OECD books, periodicals and statistical databases. Visit ***www.oecd-ilibrary.org***, and do not hesitate to contact us for more information.

OECDpublishing
www.oecd.org/publishing

ISBN 978-92-64-09543-4
97 2010 16 1 P

