Harmonisation of Regulatory Oversight in Biotechnology

Safety Assessment of Transgenic Organisms in the Environment, Volume 6

OECD CONSENSUS DOCUMENTS

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Foreword

From their first commercialisation in the mid-1990s, genetically engineered crops (also known as "transgenic" or "genetically modified" plants) have been approved for commercial release in an increasing number of countries, for planting or for entering in the composition of foods and feeds, or use in industrial processing. Up to now, the large majority of these agricultural productions remain for soybean, maize, cotton and rapeseed (canola), as outlined in *The Bioeconomy to 2030: Designing a Policy Agenda* (OECD, 2009). Despite some differences in total estimates, all analyses and statistics concur in underlining the general increasing trend in volumes produced and traded, number of countries involved and growth potential. For instance, James reports in the *Global Status of Commercialized Biotech/GM Crops: 2014, ISAAA Brief No. 49* that the surface area of transgenic crops worldwide constantly increased over the 19-year-period from 1996 to 2014, to reach 181.5 million hectares grown in 28 countries. To date, genetically engineered varieties of over 25 different plant species (including crops, flowers and trees) have received regulatory approval in OECD and non-OECD countries from all regions of the world. Such approvals for release in the environment usually follow a science-based risk/safety assessment before being granted.

The five main producers of genetically engineered crops in 2014 were the United States, followed by Brazil, Argentina, India and Canada, covering together almost 90% of the total area. Interestingly, developing countries grew more of global transgenic crops (53%) than industrial countries, at 47%. Among the 28 countries having planted those crops in 2014, only 9 of them were OECD countries, listed by decreasing area as follows: the United States, Canada, Australia, Mexico, Spain, Chile, Portugal, the Czech Republic and the Slovak Republic. In addition, some countries do not grow genetically engineered plants but import the produced commodities, for use in their feed industry in particular, as it is the case in several jurisdictions of Europe as well as some other economies worldwide.

Information on the transgenic crops which have been approved for commercial release in at least one country (for use in agriculture and/or foods and feeds processing) can be found in the OECD *Biotrack Product Database* (*www2.oecd.org/biotech*). Each transgenic product and its Unique Identifier are described, with information on approvals in countries. To date, this database covers about 240 approved genetically engineered plant varieties, and will be extended in future years to include additional species and information from a larger group of countries.

Modern biotechnologies are applied to plants, and also trees, animals and micro-organisms. The safety of the resulting genetically engineered organisms when released in the environment for their use in agriculture, food and feed industry, as biofuel or for other applications represents a challenging issue.

This is already true nowdays with the increasing cultivation of transgenic crops. It will be even more critical in the future as applications of biotechnologies widen to new

species and new areas: a growing number of novel organisms will have to be assessed before their possible use and market release. Among the ongoing developments of modern biotechnology, crop varieties modified for gaining adaptation features such as the resistance to certain biotic/abiotic stresses, result in better resilience to climate change. "Bio-fortification" (applied to rice, tuber crops and other species) develop varieties with enhanced content in some constituents, e.g. vitamins or minerals. Plants with reduced lignine or with increased oil content are examples of products sought to facilitate industrial uses of the commodities and decrease the production costs. As highlighted in the proceedings of the OECD Conference "*Biosafety and the Environmental Uses of Micro-organisms"* held in 2012, a range of new species are contemplated as potential biofuels to provide renewable energy; among them algaes, with photosynthetic cyanobacteria, are of special interest as they can be cultivated year round on non-arable land, alleviating the pressure on agricultural land and freshwater resources that would be exerted by crops growng for biofuel purposes. Less anticipated, genetically engineered mosquitos are used in few places since 2014 to control the insect population and fight tropical diseases transmitted by them. Other biotechnology developments, and in particular applied to micro-organisms, might lead to other products such biofertilizer organisms living in symbiosis in crop roots and optimising the nitrogen fixation, or biocontrol agents acting as plant protection products to control disease and attack by insects. Other exploratory fields may comprise bioremediation by using of living organisms for removing contaminants from the environment such as polluted land, or the development of detergents containing micro-organisms.

Even if it is difficult to predict which of these new biotechnology developments would lead to large applications in a medium term, it is expected that some of the products will have important impacts in their respective economic sectors. A scientifically sound approach to their risk assessment should inform biosafety regulators and support the national decisions regarding their potential release. Genetically engineered products are rigorously assessed by their developers during their elaboration, and by governments when ready for commercial use, to ensure high safety standards for the environment, human food and animal feed. Such assessments are felt essential for a healthy and sustainable agriculture, industry and trade.

An environmental safety/risk assessment of transgenic organisms is 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 (the "Working Group") 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 efforts. The biosafety consensus documents are one of the major outputs of its work.

The biosafety consensus documents constitute 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 OECD 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 the OECD community and beyond in other juridictions wishing to use them during their assessment process.

As of December 2015, a total of 53 consensus and guidance documents on biosafety have been published by the Working Group. They include documents which address the biology of plants, trees and micro-organisms as well as those dealing with specific traits that are used in genetically engineered crops. In addition, documents of broader nature aiming to facilitate harmonisati-on have been developed.

The volumes of this publication published in 2016 contain a compilation of those biosafety consensus documents issued in 2011 and 2012 (Volume 5), and from 2013 to 2015 (Volume 6). Both of them contain the "Introduction to the biosafety consensus documents" published earlier (and slightly updated since Volumes 3 and 4 of 2010). The introduction 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.

Along with previous Volumes 1-4 (OECD, 2006a; 2006b; 2010a; 2010b) the present publication offers ready access to those consensus documents published on the OECD BioTrack website thus far. As such, Volumes 5 and 6 should be of value to applicants for commercial uses of transgenic organisms, regulators in national authorities, breeders, risk assessors as well as the wider scientific community.

This biosafety work is complementary of the activities of the OECD programme on novel food and feed safety, in particular to the consensus documents developed on the composition of foods and feeds derived from transgenic organisms, which detail the key nutrients, anti-nutrients, toxicants and other constituents that can be used in a comparative approach. More information on this programme can be found in the introduction.

As each of the consensus documents may be updated in the future when 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, the OECD's other harmonisation activities. Comments can be provided to: ehscont@oecd.org.

The published consensus documents are also available individually from the OECD's Biotrack website, at no cost (*www.oecd.org/biotrack)*. Some updates have been made to data and citations in this edition.

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 chapter is composed of a "consensus document" which was prepared under the leadership of one or several countries and observer delegations, 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, whether from OECD member countries, non-member economies or observer organisations.

Each consensus document was issued individually, as soon as it was finalised and agreed on declassification, by the OECD Environment, Health and Safety (EHS) Division in the Series on Harmonisation of Regulatory Oversight in Biotechnology. Volumes 5 and 6 of this publication, containing the 2011-15 consensus documents, were prepared by Jennifer Allain and edited by Bertrand Dagallier, under the supervision of Peter Kearns, at the EHS Division, OECD Environment Directorate.

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Executive summary

This document constitutes the sixth volume of the OECD Series on Harmonisation of Regulatory Oversight in Biotechnology, which relates to the environmental risk/safety assessment of transgenic organisms, also called "biosafety". It is a compendium collating in a single volume the individual "consensus documents" published by the Working Group on the Harmonisation of Regulatory Oversight in Biotechnology. The five previous volumes of the series covered documents issued from 1996 to 2012. This Volume 6 contains the consensus documents published during the 2013-15 period.

Modern biotechnologies are applied to plants, and also trees, animals and micro-organisms. The safety of the resulting transgenic organisms when released in the environment for their use in agriculture, food and feed industry or for other applications, represents a challenging issue. This is true nowadays with the increasing cultivation of genetically engineered crops, and might become more crucial with future biotechnology developments widening to new species (e.g. insects, algae) and new targets, such as crops adapted to climate change, plants of improved composition (biofortification), products for easier processing, renewable biofuels, insects modified to prevent diseases, biofertilisers and other applications. Genetically engineered products are rigorously assessed by their developers during their elaboration, and by governments when ready for release, to ensure high safety standards for the environment, human food and animal feed. Such assessments are felt essential for a healthy and sustainable agriculture, industry and trade. The growing number of novel organisms will also need to be assessed through a scientifically sound approach to risk assessment that will inform biosafety regulators and support the decision concerning their release.

The OECD Working Group on Harmonisation of Regulatory Oversight in Biotechnology was established in 1995. It gathers national authorities responsible for the environmental risk/safety assessment of products of modern biotechnology in OECD countries and in other economies which are key stakeholders in their production and use. Observer international organisations and experts involved in biosafety are associated with this work. The Working Group's primary goals are to promote international regulatory harmonisation, to ensure that methods used in the risk assessment of genetically engineered products are as similar as possible, therefore opening the way to possible recognition and even acceptance of information from other countries' assessments. The benefits of harmonisation are multiple: it strengthens mutual understanding among countries, avoids duplication, saves resources and increases the efficiency of the risk assessment process. Overall, it improves safety while reducing unnecessary barriers to trade.

The consensus documents constitute the main output of the Working Group. They offer practical tools which compile science-based information relevant to the risk/safety assessment of transgenic organisms intended for release in the environment. They are publicly available and considered worldwide as solid references for biosafety.

In this volume, the introduction to the biosafety consensus documents presents the OECD Working Group, the key background concepts, principles and common approach prevailing in risk/safety assessment of transgenic organisms. The purpose of the consensus documents and how they are developed are also described.

Chapter 1 provides guidance and information on issues relevant to the risk/safety assessment of low-level presence (LLP) situations, which relate to seed containing small amounts of transgenic material that have been authorised for cultivation in an exporting country but not in the country of import. The availability and use of information when facing such cases is explored, with elements to consider and approaches to the management of LLP situations.

Chapter 2 deals with the biology of sugarcane (*Saccharum* spp.). This information can be a useful tool for the biosafety assessment. It contains elements of taxonomy; centre of origin; domestication and cultivation practices; morphological characteristics; reproductive biology; pollination and vegetative growth; genetics; abiotic interactions with nutrients, temperature, water and other stresses; interactions with weeds, pests and pathogens; hybridisation and introgression, and biotechnological developments.

Other crops are similarly considered and their biology described in the following chapters: Chapter 3 relates to cassava (*Manihot esculenta*), Chapter 4 to common bean (*Phaseolus vulgaris*) and Chapter 5 to cowpea (*Vigna unguiculata*). Chapter 6 deals with the biology of eucalyptus tree, being focused on those *Eucalyptus* species and hybrids which are planted commercially and expected to be the subjects of possible genetic modification aiming to improve their performance, resistance and adaptation to stressing conditions.

The set of science-based information and data contained in this volume, previously agreed by consensus and published by the OECD, constitute a solid reference recognised internationally. It is already widely used as part of biosafety assessments. As such, this publication should be of value to applicants for commercial uses of transgenic organisms, to risk assessors and regulators in national authorities in charge of granting approvals to their release in the environment, as well as the wider scientific community.

Introduction to the biosafety consensus documents

About the 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 34 member countries of the 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), 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, participation was enlarged to other non-member economies including Brazil, Bangladesh, the People's Republic of China, Colombia, India, Indonesia, Kenya, Moldova, Paraguay, the Philippines and South Africa, as well as the African Biosafety Network of Expertise from the New Partnership for Africa's Development, a body from the African Union (AU-NEPAD-ABNE). From July 2011 to December 2014, a programme was jointly implemented by the World Bank, the ILSI Research Foundation – Center for Environmental Risk Assessment (ILSI-CERA) and the OECD in the framework of the "Partnership for Biosafety Risk Assessment and Regulation", which developed new links, enhanced collaboration and supported the participation of four non-member economies in the activities of the Working Group.

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, is as similar as possible. It could lead to countries recognising or even accepting information from one another's' 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).

The need for harmonisation activities at the 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 the 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 for Environmental Aspects of Biotechnology took into consideration, and built upon, the earlier work at the 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 the annex to this introduction.)

Key background concepts and principles

The Ad Hoc Group for Environmental Aspects of Biotechnology 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, 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 the annex 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 for Environmental Aspects of Biotechnology 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 an initial group of 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.

A common approach to risk/safety assessment

An important aspect for the Ad Hoc Group for Environmental Aspects of Biotechnology 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 is 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; 1995d). 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/guidance texts) 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: 1) general scientific considerations; 2) human health considerations; and 3) 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.

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 recognised that biology and trait consensus documents could be agreed upon relatively quickly by member countries (within a few years). This compilation process was quickly formalised in the drafting of consensus documents.

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 consensual 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: 1) by applicants as information to be given in applications to regulatory authorities; 2) by regulators as a general guide and reference source in their reviews; and 3) 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 member countries, though the precise meaning of these terms is still open for discussion. During the period of the Ad Hoc Group for Environmental Aspects of Biotechnology and the early days of the Working Group (1993-95), 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 a 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 assessments.

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 it 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 can 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 activities relating to tropical and subtropical species. 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 the 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 *Oryza sativa* (rice) published in 1999.

The full series of consensus documents developed by the Working Group is also published in compendium documents, as it is the case for these volumes 5 and 6 issued in 2016. Previous volumes 3 and 4 were published in 2010 (covering 2007-10), while volumes 1 and 2 were issued in 2006 (covering1996-2006) (OECD, 2010b; 2010c; 2006a; 2006b).

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 considers 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 guidance document on “Points to consider” for consensus documents on the biology of cultivated plants that was published in 2006, and then that of the trait documents. The “Points to consider’ document, included in Volumes 3 and 4 of the compendia series, is currently under review by the Working Group to update it with the latest developments.

Within the important ongoing activities of the Working Group, a new document is being developed on the “Environmental considerations for the risk/safety assessment for the release of transgenic plants”. Focused on the core of the biosafety work that is applied to crops and trees, and taking into account the most recent views from countries of all regions of the world, this document will constitute a key guidance tool for developers, assessors and regulatory authorities. It is expected to be published around 2017.

Other projects are implemented to prepare consensus documents on the biology of animals, to date on the Atlantic salmon (*Salmo salar*), and on the mosquito *Aedes aegypti*, for which some genetically engineered strains are used since 2014 in limited areas to control the virus-vector insect population and participate in the fight against the tropical

diseases such as dengue fever and chikungunia that have been dramatically extending in many regions of the world over the last decade.

The Working Group is also considering projects on micro-organisms, therefore opening to new areas, for instance, bioenergy, with the preparation of a document on eukaryotic micro-algae having started recently. The photosynthetic cyanobacteria are potential providers of renewable energy and are of special interest as they can be cultivated year round on non-arable land, alleviating the pressure on farmland and freshwater resources that would be exerted by crops grown for biofuel purposes, as stated in the proceedings of the OECD Conference on Biosafety and the Environmental Uses of Micro-Organisms set up by the Working Group in 2012 (OECD, 2015a). Other biotechnology developments applied to micro-organisms might be considered to prepare future documents: updated review of biofertilizer organisms living in symbiosis in crop roots and optimising the nitrogen fixation, or biocontrol agents acting as plant protection products to control disease and attack by insects and other herbivores. Other exploratory fields may comprise bioremediation by using living organisms for removing contaminants from the environment such as polluted land, or the development of detergents containing micro-organisms.

In recent years, the Working Group started to exchange knowledge and promote discussion on the new plant-breeding techniques and their potential impact of risk/safety assessment. An OECD workshop was organised on these matters by the Working Group in 2014, and the report will be published soon.

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 assessment, 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's 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, 26 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 (*www.oecd.org/env/ehs/biotrack*).

The full series of consensus documents developed by the Task Force was published in 2015 in two compendium documents, Volume 1 covering 2002-08 and Volume 2 covering 2009-14 (OECD, 2015b; 2015c).

The Working Group and the Task Force are implementing closely related and complementary programmes, focused on environmental aspects for the first and on food and feed aspects for the second. Their co-operation on issues of common interest resulted

in the first document developed jointly by the two bodies, the "Consensus document on molecular characterisation of plants derived from modern biotechnology", published in 2010 (included in Volume 3 of the current series).

Notes

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 for Environmental Aspects of Biotechnology 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.
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 – Volumes 5 and 6 – the Chair is from the United States, and the Vice-Chairs from Australia, Belgium, Finland, Japan and Mexico.

Annex: OECD biosafety principles and concepts developed prior to the Working Group on Harmonisation of Regulatory Oversight in Biotechnology (1986-94)

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 on Harmonisation of Regulatory Oversight in Biotechnology, the 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 annex 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, the 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* (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. The OECD published general principles for scale-up (OECD, 1993a), which reaffirmed 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 (US-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 (US-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 effect 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).

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Part I:

Facilitating harmonised safety assessments

Chapter 1.

Low-level presence of transgenic plants in seed and grain commodities: Environmental risk/safety assessment and availability and use of information

This chapter provides guidance and information on the environmental risk/safety assessment of low-level presence (LLP) situations. LLP relates to seed containing small amounts of transgenic material that have been authorised for cultivation in an exporting country, but not in the country of import. It covers agricultural seed used for planting, and commodities (e.g. grains and oilseeds) that can grow into plants when unintentionally released in the environment or used for cropping. This chapter discusses the availability and the use of information when facing LLP situations: LLP occurrences in seed, national systems dealing with them, principles for determining environmental risk/safety for transgenic plants, data and information available to perform such an assessment, with examples of scenarios. Approaches to the management of LLP situations and possible ways to proactively address their environmental safety are considered.

This chapter was prepared by the OECD Working Group on the Harmonisation of Regulatory Oversight in Biotechnology, with the Bureau of the Working Group having served as lead. It was initially issued in September 2013, together with replies from 20 countries and observers to a questionnaire on experiences with LLP situations (OECD, 2013).

Foreword

The major output of the Working Group on the Harmonisation of Regulatory Oversight in Biotechnology over the years has been its consensus documents. These documents contain information for use during the regulatory assessment of a particular product. In the area of plant biosafety, consensus documents are published on information on the biology of certain plant species, selected traits that may be introduced into plant species, and biosafety issues arising from certain general types of modifications made to plants.

The scope of this chapter is different from that of the consensus documents. It covers low-level presence situations in which seed (or certain commodities) contain low levels of transgenic seed that have been reviewed for environmental risk/safety and received authorisation for commercial cultivation (unconfined release) in one or more exporting countries but not in the country of import.

The Bureau of the Working Group on the Harmonisation of Regulatory Oversight in Biotechnology took the lead in preparing this chapter, and the draft has been revised on a number of occasions based on the input from other member countries and stakeholders.

Preamble

The OECD's Working Group on the Harmonisation of Regulatory Oversight in Biotechnology (hereafter referred to as the "Working Group") has since its inception in 1995 developed technical documents that facilitate environmental risk/safety assessment of transgenic[1] organisms, especially plants. These tools for risk assessors and regulators include science-focused documents on the biology of the organism and introduced traits, documents that supplement and expand upon the information in the biology and trait documents (e.g. module II on herbicide tolerance; OECD, 2002), and guidance documents (e.g. how to use information from detection technologies for bacteria; OECD, 2004) and a document on molecular characterisation of transgenic plants (OECD, 2010). In effect, a suite of documents has been developed concerning the environmental review of the products of modern biotechnology.

The environmental risk/safety assessment of transgenic organisms is based on 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 (OECD, 1993a; 1986). The OECD's Working Group decided at its first session to focus its work on identifying parts of this information which could be commonly used by countries for environmental risk/safety assessment to encourage information sharing and prevent duplication of effort among countries. The trait and biology biosafety consensus documents are one of the major outputs of its work. They are intended to be a "snapshot" of current information on a specific host organism or trait, for use during environmental risk/safety assessments. They address the key or core set of issues that member countries believe are relevant to environmental risk/safety assessment. 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. This information is said to be mutually acceptable among member countries. To date, 53 biosafety consensus documents have been published.[2]

In addition to the biology and trait biosafety consensus documents, the Working Group also takes on important emerging issues related to environmental risk/safety

assessment and regulatory harmonisation. Each of these projects is different from the biosafety consensus documents and from each other. Examples of such projects include the "Consensus document on molecular characterisation of plants derived from modern biotechnology" (OECD, 2010), "OECD guidance for designation of a unique identifier for transgenic plants" (OECD, 2000; 2006b); and "Points to consider for consensus documents on biotechnology of cultivated plants" (2006a). In 2007, the topic of information availability and sharing and possible guidance for environmental risk/safety assessment of low-level presence of unauthorised transgenic plant material in seed in cases where such an assessment had been carried out in at least one country was proposed as a project for the Working Group. In 2008, a workshop to explore the topic was held in Paris and subsequently the Working Group agreed to develop a project proposal. This project was to align with the remit of the Working Group, whose terms of reference focus on scientific and technical aspects. Finally, in 2009, the Working Group agreed to the project and at this time commodities were added to the scope. The final scope of the document is identified as follows:

> **The scope of this document covers low-level presence situations where […] seed contain low levels of transgenic seed that have been reviewed for environmental risk/safety and received authorisation for commercial cultivation (unconfined release) in one or more exporting countries but not in a country of import. […]** This document covers commercial seed used intentionally for planting as well as commodities (e.g. grains and oilseeds) that can germinate and grow into plants when unintentionally released into the environment during handling and transport or when intentionally used for planting (OECD, 2013).

A questionnaire was circulated late in 2009 to gather information on participant countries' experiences with low-level presence (LLP) situations in seed and certain commodities to use as a basis for this chapter. Twenty participant countries (OECD member and non-member countries) and observers responded to a questionnaire on their experience in addressing LLP situations: Argentina, Australia, Austria, Belgium, Brazil, Canada, Chile, Czech Republic, Estonia, Japan, Korea, Mexico, the Netherlands, New Zealand, Norway, the Philippines, Spain, Turkey, the United States and the Business Industry Advisory Committee to the OECD (BIAC). In addition, the information in this chapter was obtained from extensive discussions within the Working Group and the dedicated workshop that took place in April 2008.

In developing this chapter, the Working Group discussed the possibility of taking the same approach as the Codex Alimentarius by linking the discussion of LLP to an existing text on environmental risk/safety assessment. Focused on food safety, the Codex Alimentarius has an annex addressing LLP as part of its *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants* (Codex Alimentarius Commission, 2003). The annex illustrates how to use the guideline as it would apply to an LLP situation in food based upon different predicted exposure scenarios. Similar to the Codex Alimentarius LLP annex, this chapter focuses on transgenic plants that have been reviewed for risk/safety in one or more countries and occasionally are present in importing countries in which the risk/safety of the relevant recombinant-DNA plants has not been determined. However, the LLP situations discussed are not in food or feed but in seed and grain commodities that can function biologically as seed, and the concern is environmental risk/safety rather than food safety. LLP situations in seed and commodities are discussed in the context of the paradigm for environmental risk/safety assessment that has been articulated in the OECD scale-up document (OECD, 1993a) and elsewhere.

Executive summary

Modern trade and agriculture are characterised by the increasing exchange worldwide of agricultural commodities, including seed. Many countries import and export significant quantities of seed for sowing, as well as grain and oilseed commodities that can function biologically as seed if released into the environment. A feature of modern agriculture is the increased use of transgenic plants. Since the mid-1990s, the adoption of transgenic plants has increased in the numbers developed, the volumes grown and the number of countries where such plants are grown. This increase in the development and use of transgenic plants occurs within the context of the continued use of the many crop plant varieties developed using conventional breeding techniques and the increasing exchange worldwide of seed and other propagules as well as viable grain and oilseed commodities.

Many countries have national legislation that addresses the need for regulation of the use of transgenic plants and most countries require prior domestic authorisation involving an environmental risk/safety assessment before unconfined release into the environment (i.e. commercial cultivation) of such plants is allowed. Authorisations for commercial cultivation in each jurisdiction generally occur independently of other countries. At any given time, there may be transgenic plants authorised for commercial cultivation (unconfined release) in one country that have not been authorised in other countries with which the authorising country trades seeds and commodities. This is often referred to as "asynchronous" authorisation. Such asynchrony can occur because the timing of the authorisation process is different between countries or possibly because authorisation is never sought from or granted by one or more of the countries involved in seed and/or grain importing activities.

Aggregation and mixing in crop production or trade along with biological factors such as cross-pollination between crops can result in situations where traded seed or commodity lots contain unintended low levels of transgenic seed authorised in one or more exporting countries but not in an importing country due to asynchronous authorisation. The scope of this chapter covers low-level presence (LLP) situations where these seed contain low levels of transgenic seed that have been reviewed for environmental risk/safety and received authorisation for commercial cultivation (unconfined release) in one or more exporting countries, but not in a country of import.

This chapter covers commercial seed used intentionally for planting as well as commodities (e.g. grains and oilseeds) that can germinate and grow into plants when unintentionally released into the environment during handling and transport or when intentionally used for planting. In this chapter "seed" refers to both seed and commodities. LLP situations in seed may potentially occur and be detected before planting, in plants in the field or in some cases along transport routes after a commodity grown from seed has been harvested. It is anticipated that the number of such LLP incidents is likely to increase globally (Stein and Rodriguez-Cerezo, 2009) because of increasing numbers of transgenic seeds entering the market and the increasing international movement of seeds and/or commodities.

The issue of LLP in seed raises questions regarding environmental risk/safety and compliance with mandated legislative requirements for the importing country as well as the seed and commodity trade industries in both importing and exporting countries. An environmental risk/safety assessment may be undertaken by the importing country to evaluate the environmental risk/safety of the unauthorised transgenic plant in the LLP situation, not for the purpose of authorisation but rather, the assessment can provide

a basis for, and may be used by, the importing country to inform decisions to mitigate and/or manage the LLP situation. This chapter presents approaches to information availability and sharing and risk/safety assessment in LLP situations where there is knowledge of the identity of the unauthorised transgenic plant.

Major considerations in responding to an LLP situation are likely to be managing any environmental risks and returning the situation to compliance with relevant legislation. The response by an importing country to an LLP situation can vary, depending upon the situation itself and the legal framework under which the country operates. Ultimately, legislative requirements will provide the underpinning for decisions by a national authority, including mitigation or other actions taken to address the potential environmental risk/safety of an LLP situation.

Many OECD countries have already had experience with LLP in seed and there is much value to be gained from sharing and understanding this experience. This chapter captures the experience of the participant countries of the OECD Working Group for the Harmonisation of Regulatory Oversight in Biotechnology in addressing asynchronous authorisation LLP situations in the environment, particularly with regard to information availability and sharing, and to the scientific basis and approach for undertaking an environmental risk/safety assessment in an LLP situation. This chapter does not prescribe how national authorities should manage incidents of LLP, take decisions, or define what LLP is or what proportion of unauthorised transgenic seeds constitutes a LLP situation (e.g. threshold) under their own legislative framework.

One of the aims of this chapter is to serve as an aid to risk assessors and regulators, providing guidance on handling the aspects of an environmental risk/safety assessment and accessing and using information in an LLP situation where there is asynchronous authorisation of the transgenic plant involved. Strategies to do an adequate risk/safety assessment in an LLP situation are discussed as well as how best to proceed in circumstances where a less than full information set may be available so that an importing country can still expeditiously determine appropriate mitigation measures for addressing the LLP situation in a manner commensurate with the risk presented. This chapter is a compilation of current approaches to environmental risk/safety assessment, information access and information use in addressing LLP situations in seed and includes examples of how such an assessment may be used to inform environmental risk management and returning an LLP situation to compliance with legislative mandates. This chapter can be used as guidance in addressing an LLP situation in seed in combination with other OECD documents related to environmental risk/safety assessment such as the trait, biology and molecular characterisation documents (see OECD BioTrack website)[2].

Principles for determining environmental risk/safety for transgenic plants

The general principles for determining risk/safety are the same for an LLP situation in the environment as they are for an authorisation of a transgenic plant for unconfined release. These principles are articulated in the OECD *Safety Considerations for Biotechnology: Scale-up of Crop Plants* (OECD, 1993a), which describes risk analysis[3] as being: "based on the characteristics of the organism, the introduced trait, the environment into which the organism is introduced, the interactions between these, and the intended application". The "[k]nowledge of and experience with any or all of these provides familiarity which plays an important role in risk/safety analysis […] Familiarity is not synonymous with safety; rather, it means having enough information to be able to

judge the safety of the introduction or to indicate ways of handling the risks". These principles apply in the following ways to an LLP situation:

1. available knowledge and experience can guide the risk assessment
2. an environmental risk/safety assessment can be used to evaluate potential risks to the environment in a particular LLP situation
3. use of information and understanding from previous assessments of the same or similar plant, both domestically and in other countries, may inform the assessment.

Availability of data and information to perform a risk/safety assessment in an LLP situation

In LLP situations, national authorities face numerous challenges, including that relevant data for the environmental risk/safety assessment may be lacking because it is either not immediately available (e.g. in an application) or inadequate. Typically, the amount of data and information available in an LLP situation may not in the short term be equivalent to that which would be available from an application for full authorisation of the transgenic plant for cultivation. Information sharing between countries may be important in LLP situations where addressing the situation might be facilitated with expedited access to information. The importance of collaborative working relationships between national authorities in different countries cannot be over-emphasised. Data and information regarding relevant characteristics of the plant, the behaviour of the plant in the environment, including cultural practices, and the trait may be available from a variety of sources. Two obvious sources of information are: 1) that developed for assessment in the country in which the transgenic plant was authorised prior to export; and 2) that submitted to regulators for assessment in the importing country. However, even if information is available from either or both of these two sources, the risk assessor may still need to actively and rapidly access information from additional sources to obtain sufficient information to make an assessment of the environmental risk/safety.

Some of the information needed to adequately evaluate the environmental risk/safety of an LLP situation in seed can come from existing knowledge and experience with: 1) the same non-modified plant species or similar closely-related plant species; 2) the known functions(s) of the same or similar gene and/or its expression products (e.g. protein); 3) the effect of the same or similar phenotype or trait in plants on the environment; and/or 4) the same or similar receiving environments. Much information may already be available from existing reviews of the same or similar plants within a country or in other countries.

Access to the required information can be facilitated by the use of Internet databases listing authorisations. Because the usefulness of these sources is dependent on their content and currency, it is important for countries to keep their information updated in these databases to maximise their usefulness. To enhance information sharing between countries, the OECD BioTrack website (*www.oecd.org/biotrack*) provides information on biotechnology regulatory contacts for OECD and participating countries, including information on regulatory frameworks and access to OECD biology and trait documents. Knowledge of the OECD Unique Identifier of a transgenic plant can facilitate access to information in the OECD BioTrack and other databases (OECD, 2006b).

Environmental risk/safety assessment in an LLP situation

When approving a transgenic plant for potential cultivation, usually the environmental risk/safety assessment assumes 100% exposure over an extended period of time, i.e. the plant is cultivated on potentially very large areas of land. This is an assessment of a product for intentional use. However, when assessing an LLP situation, the context may be different. The determination of environmental risk that an unauthorised transgenic plant may pose is based not only on the hazards identified, but on the potential exposure which will be related to the scale of an LLP situation. The amount and degree of information needed may be different for an LLP situation because of the reduced scale and the purpose of the assessment. By definition, generally, an LLP situation is at a scale reduced from that assumed present in a risk/safety assessment for authorisation for large-scale cultivation of the same plant. In an LLP situation, the environmental risk/safety assessment is not intended to lead to an authorisation. However, the results of the assessment can be useful in supporting environmental risk management decisions through scientifically evaluating potential options for managing any risk identified.

Available knowledge and experience, data and information, about the scale (i.e. amount of seed distributed spatially and temporally) of the LLP situation, the trait, and the plant and the receiving environment of the importing country can facilitate a rapid environmental risk/safety assessment of an LLP situation. While this guidance does not explain explicitly how to do such an assessment, it is noted that the types of information used are generally the same as for the review of an application for authorisation where much of the information is supplied in the application itself. There is ample discussion of these types of information and their importance to environmental risk assessment in previous OECD publications (OECD, 1993a; 1993b; 1992). The basic safety issues that may potentially be of concern were also identified in these publications.

The majority of LLP situations to date have involved “common” crop species and trait combinations that have been widely adopted and are under large-scale cultivation where authorised. There is substantial knowledge and experience with these crop species as they are grown regularly within the countries in which LLP situations have occurred, as non-transgenic or transgenic crops. The available broad domestic or global experience and knowledge of how the major traits being used today, particularly the herbicide-tolerant and insect-resistant traits, affect different plant types in different environments may provide a range of possibilities of how the trait may affect the behaviour of the plant in the environment of a particular LLP situation.

Familiarity with the biology of the crop plant and its behaviour in the receiving environment in the context of the existing agricultural practices (cultivation and environmental management) of the country or region can be used to identify aspects of the environment that may potentially be affected in an LLP situation. Previous assessments of the same or a similar plant that have addressed what potential adverse effects might be predicted for the unauthorised transgenic plant can contribute to a rapid understanding of whether the LLP situation might result in any adverse effects. More or less information will be needed, depending upon the particular LLP situation, how quickly decisions are needed, and the core information and comprehensiveness of that information needed to take those decisions. This can facilitate rapid assessment of potential environmental risk presented by an LLP situation in seed, along with the ramifications for mitigation or risk management of the situation. The importing country with the LLP situation may use this understanding to identify the unique or different

aspects of its country/region compared to the exporting country (and other countries) where assessments for authorisation of the transgenic plant have already been completed.

Risk profile

When an environmental risk/safety assessment of an LLP situation is undertaken, the goal is to determine any risk presented and to scientifically evaluate potential options for managing such risks. In a relatively short period of time, the identity of the unauthorised plant material may need to be confirmed, the potential for adverse effects be determined and actions taken to minimise any identified environmental risk presented by the LLP situation. A risk profile characterising the situation may be rapidly assembled based upon data and information from reviews of the same or similar authorised plants and/or existing knowledge and familiarity with the plant, trait, environment and their interaction. The risk profile recognises the scale of the LLP situation and may expeditiously inform decisions to manage or mitigate any risk presented as well as to return the situation to regulatory compliance.

The following process can be used to develop a risk profile to expeditiously address an LLP situation in the environment of the importing country subsequent to identification of the presence of an unauthorised transgenic plant:

- determine where the LLP situation has been found in the environment and the potential distribution of the unauthorised transgenic plant
- identify relevant sources of information, including previous assessments of that unauthorised transgenic plant available either domestically, regionally or from other countries
- determine if those assessments identified any potential hazards and whether/how these relate to the importing country's protection goals and could potentially affect the receiving environment harbouring the unauthorised transgenic plant
- determine/consider whether there are pathways for distribution of the unauthorised transgenic plant in the LLP situation through which the identified hazard can cause adverse effects in the receiving environment
- assess the likelihood and consequence of those adverse effects being realised.

A risk profile can characterise the risk that may occur or has occurred given the specifics of the LLP situation (case-by-case). The environmental risk/safety assessment may include an evaluation of management options for any risk to the environment that might be presented, such as an evaluation of existing or modified distribution systems and agricultural practices used with the particular plant species. The assessment can also provide the needed scientific basis to inform broader management objectives, such as those to return the situation to compliance with regulatory requirements. In the context of this discussion, such management options may include mitigation of any further release of unauthorised plants into the environment and/or remediation of any release that has already occurred.

Uses of an environmental risk/safety assessment in the management of LLP situations in seed

The "Scale-up" document (OECD, 1993a) describes environmental risk management as "the way appropriate methods are applied in order to minimise scientifically identified risks … In principle, appropriate management is based on and should be in proportion to

the results of the risk/safety" assessment. "Risk management encompasses all aspects of the management of the organism indirectly through management of the environment into which the organism is introduced, or directly, by management of the organism itself."

In general, management of an LLP situation may focus on the goals of protecting the environment (environmental risk management) and/or returning the situation to compliance with the requirements of a country's legislative framework.

An environmental risk/safety assessment may be useful in informing decisions for environmental risk management and returning the LLP situation to compliance with the regulatory requirements of the country or region, recognising that the use of an environmental risk/safety assessment for this purpose may depend on the provisions of the legislative framework of the country. The form that management of the LLP situation takes can be influenced by multiple factors. The complexity of the response may be influenced by, for example, socio-economic factors, legislative mandates, stakeholder preferences or the availability of resources. In some cases, an environmental risk/safety assessment may not be needed to address a particular LLP situation due to the adoption of processes to handle an LLP. Or, alternatively, the legislative framework may stipulate that LLP situations must be returned to compliance regardless of whether or not an environmental risk/safety assessment is performed.

Depending on the country's legislative framework, an environmental risk/safety assessment can provide options for environmental risk management in a manner proportional to any risk presented to achieve protection goals (OECD, 1993a). The concept of risk management measures being proportional to the level of risk presented as determined by a risk assessment is consistent with internationally accepted risk management principles.

As part of an approach to managing an LLP situation overall, an environmental risk/safety assessment can be used to characterise the situation, including identifying any environmental risk associated with the situation and the measures either in place or needed to manage any such risk presented; it may also suggest the most efficacious measures to return the situation to compliance with legislative mandates. The circumstances and timeframe of an LLP situation in seed is a major factor for determining the appropriate environmental risk management/mitigation measures, depending upon the risk presented – e.g. removal or destruction of the unauthorised transgenic plants prior to flowering may or may not be important in limiting potential spread or persistence. In addition, the same measures may contribute to returning the situation to compliance with legislative mandates; e.g. remediation and mitigation options that ultimately lead to limitation of the maintenance and/or spread and/or removal of the unauthorised plant from the environment and ultimately the seed supply. The situation and the assessment can indicate options for disposal of the plant material in a manner that is proportional to the risk identified, returns the situation to compliance and does so in a manner that is least disruptive to the agricultural system.

While it is up to each country, considering its legislative framework, to decide on appropriate management strategies, options other than crop or seed destruction may be considered when attempting to manage the LLP situation in a manner that is proportional to the risk identified and the need to return the situation to compliance. For some countries, it may not be feasible to implement some of the options, as their application may be governed by the legislative framework of the particular country. For many countries, an LLP situation is almost, by definition, a situation of non-compliance with

regulatory requirements and in many jurisdictions there are legal requirements for compliance that also set the context for any management for environmental risk.

Potential ways to proactively address environmental risk for LLP situations

Given that the incidence of LLP situations resulting from asynchronous authorisation is anticipated to increase globally (Stein and Rodriguez-Cerezo, 2009) and that such situations have the potential to be disruptive to trade and create economic hardship on seed producers, importers, shippers and farmers as attested in responses to the OECD questionnaire (Annex 1.A1), countries and regions have taken several approaches to proactively address LLP situations. Some of these approaches focus on steps to limit the potential for uncertainty regarding environmental risk. Others attempt to work with industry to limit the potential for the occurrence of an LLP, and still others establish procedures to facilitate a rapid response to an LLP situation.

Most countries have not developed explicit rules or policies to address LLP situations in the environment. However, a few have published policies and guidelines or elaborated more general strategies to limit the occurrence of unauthorised transgenic plants in the environment, including those arising from LLP situations. These policies and plans serve to communicate to the public the government's approaches to dealing with potential environmental risk from LLP situations and to clarify the responsibilities of various stakeholders, including potential industries involved (e.g. seed production, breeding, trading, transport) in order to limit, as well as prepare for, a potential occurrence of an LLP in the environment.

Introduction

Modern trade and agriculture are characterised by the increasing exchange worldwide of agricultural commodities, including seed. Many countries import and export significant quantities of seed for sowing, as well as grain and oilseed commodities that can function biologically as seed once released into the environment. A feature of modern agriculture is the increased use of transgenic plants. Since the mid-1990s, the adoption of transgenic plants has increased in the numbers of plants developed, the volumes grown and in the number of countries where such plants are grown. This increase in the development and use of transgenic plants occurs within the context of the continued use of the many crop plant varieties developed using conventional breeding techniques and the increasing exchange worldwide of seed and other propagules as well as viable grain and oilseed commodities.

Many countries have national legislation that addresses the need for regulation of the use of transgenic plants and most countries require prior domestic authorisation involving an environmental risk/safety assessment before unconfined release into the environment (i.e. commercial cultivation) of such plants is allowed. Authorisations for commercial cultivation in each jurisdiction generally occur independently of other countries. At any given time, there may be transgenic plants authorised for commercial cultivation (unconfined release) in one country that have not been authorised in other countries with which the authorising country trades seeds and commodities. This is often referred to as "asynchronous" authorisation. Such asynchrony can occur because the timing of the authorisation process is different between countries or possibly because authorisation is never sought from or granted by one or more of the countries in seed- and/or grain-importing activities.

As a result of these trends and biological factors such as cross-pollination, as well as aggregation and mixing of commodity lots in trade, imported seeds or certain commodities may inadvertently contain low levels of transgenic seed that have been reviewed for environmental risk/safety and received authorisation for commercial cultivation (unconfined release) in one or more exporting countries but not in the country of import. For the purposes of this chapter, "seed" refers to both seed and commodities and such an occurrence as referred to above is called a low-level presence (LLP) situation.

The issue of LLP in seed concerns importing countries as well as the seed and commodity traders in both importing and exporting countries.

In an LLP situation in seed, a primary question may be that of the environmental risk/safety of the unauthorised transgenic plant in the country of import. Consequently, an environmental risk/safety assessment may be undertaken by the importing country to evaluate the environmental risk/safety of the transgenic plant in the LLP situation. It is important to note, however, that the intent of the assessment is not an authorisation of the transgenic plant that is present at a low level in seed or commodities. Rather the assessment can provide a basis for, and may be used by the importing country, to inform decisions to mitigate and/or manage the situation. A major consideration in managing an LLP situation is likely to be returning the situation to compliance with relevant legislation. Ultimately, legislative requirements will provide the underpinning for decisions by a national authority, including mitigation or other actions taken to address any environmental risk presented by an LLP situation.

In an LLP situation in seed, an importing country may not have had the opportunity to complete an evaluation as to whether the unauthorised transgenic plant could negatively affect the importing country's environment and the country will need to comply with its relevant legislation. This means that an LLP situation in the environment can, in many cases, require the expeditious performance of an environmental risk/safety assessment. This chapter will discuss strategies to do an adequate risk/safety assessment in an LLP situation.

Purpose and scope

The scope of this chapter covers a situation where seed contains low levels of transgenic seed that have been reviewed for environmental risk/safety and received authorisation for commercial cultivation (unconfined release) in one or more countries but not in the country of import. This chapter is to serve as an aid to risk assessors and regulators conducting an environmental risk/safety assessment and accessing and using information in response to LLP situations in seed where there is asynchronous authorisation of the transgenic plant involved. It is anticipated that the number of such LLP incidents is likely to increase globally (Stein and Rodriguez-Cerezo, 2009) because of increasing numbers of transgenic seeds entering the market, the increasing international movement of seeds and/or commodities and biological factors (e.g. inadvertent cross-pollination between seed production fields). In such LLP situations, there may be an actual or potential release of the unauthorised transgenic seed into the environment, necessitating an environmental risk/safety assessment. This chapter is intended to provide guidance on handling the aspects of an environmental risk/safety assessment in an LLP situation where there is asynchronous authorisation of the transgenic plant involved. It is a synthesis of current approaches to environmental risk/safety assessment, information access and information use in addressing LLP

situations in seed and includes examples of how such an assessment may be used to inform environmental risk management and returning an LLP situation to compliance with legislative mandates.

This chapter can be used in combination with other OECD documents related to environmental risk/safety assessment, such as the consensus documents, which address the biology of specific plant species or traits used in transgenic plants. The OECD document on molecular characterisation may also be relevant (see OECD, 2010).

This chapter covers commercial seed used intentionally for planting as well as commodities (e.g. grains and oilseeds) that can germinate and grow into plants when unintentionally released into the environment during handling and transport or when intentionally used for planting. Except where indicated, in this chapter the term "seed" refers to seed intended for planting as well as commodities that can function biologically as seed when released into the environment. LLP situations in seed may potentially occur and be discovered before planting, in plants in the field or in some cases along transport routes after a commodity grown from seed has been harvested.

This chapter presents approaches to risk/safety assessment in LLP situations where there is knowledge of the identity of the unauthorised transgenic plant. It does not, however, address the question of how to establish the identity of the unauthorised transgenic plant. In addition, this chapter does not address LLP situations arising from field trials for product development or basic research, or situations in which no authorisation has been granted in any country, although the approach described here may be fruitfully applied in such situations. This chapter also does not address issues related to food/feed safety.

Many OECD countries have already had experience with LLP in seed and there is much value to be gained from sharing and understanding this experience. While each LLP situation may manifest differently and is likely to be handled on a case-by-case basis by the importing country or region in which the situation occurs, there is benefit in identifying available sources of information and useful environmental risk/safety assessment strategies that may assist in addressing these situations. This chapter describes approaches for appraising risk/safety expeditiously (e.g. the plant is already in the environment) in circumstances where a less than full information set may be available so that the national authority can rely on the assessment to determine appropriate mitigation measures for addressing the LLP situation in a manner commensurate with the risk presented. Typically, the amount of data and information available to the assessor in an LLP situation may not in the short term be equivalent to that available from an application for full authorisation of the plant for cultivation. However, much information may already be available from existing reviews of similar plants within a country. In addition, information sharing among authorities may be important in LLP situations where addressing the situation might be facilitated with expedited access to information.

This chapter captures the experience of the participant countries of the OECD Working Group in addressing LLP situations in the environment, particularly with regard to the scientific basis and approach for undertaking an environmental risk/safety assessment in an LLP situation in the environment (individual country experiences are captured in Annex 1.A1 of the original issue, which includes references to national or regional guidance documents; see OECD, 2013). As an aid to regulators and risk assessors, the following aspects are covered:

- the occurrence of LLP situations in seed in OECD participant countries

- the types of information that can be used in an environmental risk/safety assessment and where these may be available
- how an environmental risk/safety assessment can be approached, particularly how existing knowledge and experience (familiarity) regarding the plant, trait, environment and their interactions may aid in performing an assessment
- whether and how an environmental risk/safety assessment may influence risk mitigation and management as well as the overall management of the situation.

The following points summarise and clarify how this chapter is intended to be appropriately used. This chapter:

- encompasses seed that contains low levels of transgenic seed that have been reviewed for environmental risk/safety and received authorisation for commercial cultivation (unconfined release) in one or more countries but not in the country of import
- relates to LLP in seed including commodities that can function biologically as seed
- highlights the importance of information sharing, experience and environmental risk/safety assessment in an LLP situation.

On the other hand, this chapter acknowledges national legislation and does not, amongst other things:

- preclude a national or regional authority from undertaking or not undertaking an environmental risk/safety assessment for authorisation of the transgenic plant present at low levels within the context of its regulatory system
- prevent countries from abiding by existing international agreements on the topic of LLP (i.e. the Codex Alimentarius Commission, 2003)
- prescribe how national authorities should manage incidents of LLP, take decisions or define what an LLP is under their own legislative framework
- prescribe what proportion of unauthorised transgenic seeds constitutes an LLP situation (e.g. threshold)
- address issues of food/feed safety, low-level presence arising from field trials for product development or basic research, or situations in which no authorisation has been granted in any country.

Information availability, information use and environmental risk/safety assessment in low-level presence situations

Occurrence of low-level presence in seed

The LLP of unauthorised transgenic seed may originate from a range of biological or non-biological causes during seed production of plant varieties, and the production of some commodities. It may occur during commercial cultivation, handling, harvest, transport, shipment, etc. of seed, as well as of commodities. Commercial seed for intentional planting is produced to meet certain quality standards (viability, germination, purity, etc.) while commodities, grain harvested for food, feed or processing, are not intended to meet seed quality standards as they are not normally intended for planting.

In the development of new varieties, plant breeding may occasionally result in low-level mixing of genetic material from unintended plant sources. This is true for both conventionally bred plants as well as for transgenic plants. This mixing may also occur during seed production of any variety. The potential for such low-level mixing has been formally recognised through allowances for a set maximum level of "off types" in seed certified for purity (OECD, 2016). However, with transgenic seed there is an increased need to avoid cross-pollination and ensure adequate quality control, as these can result in an LLP situation. LLP in seed can also occur via non-biological causes through commingling or mislabelling of transgenic seed. Commodities can have additional sources of unintended mixing that can lead to an LLP situation during handling, storage and transport after harvest. Given the complexity of the agricultural system, it may be very difficult to determine the actual initial cause of any particular LLP situation in either seed or commodities.

In 2014, 28 countries grew a total of 181.5 million hectares of crops that were transgenic and the majority of these contained herbicide tolerance and/or insect resistance traits (James, 2015). As a result, most of the LLP situations to date relating to the presence or release into the environment of unauthorised transgenic plants have occurred with common crop plant species and trait/gene combinations that have been reviewed by many countries. These LLP situations have included those with the commodity crops corn, cotton, rapeseed/canola and soybean containing herbicide tolerance (glyphosate or glufosinate ammonium) and/or insect resistance (*Bacillus thuringiensis* delta endotoxins effective against coleopteran or lepidopteran insect pests) traits.

Even though the plant and trait/gene combinations in these LLP situations may have been reviewed in one or more countries, the transgenic plants involved may not have been authorised for environmental release amongst all trading partners. In addition, there can be an asymmetry in the types of authorisations in the importing country compared to the exporting country; such asymmetry may occur because the national authorities either receive requests for, or grant authorisation for, different uses. The following are examples of such situations[4] that can occur in an importing country:

- no application has been received requesting authorisation either for importation for food and/or feed use or for environmental release (cultivation) of the transgenic plant or
- no authorisation has been granted though applications may have been received for food and/or feed use and/or possibly for environmental release or
- authorisation has been granted for importation for food and/or feed use, but not for environmental release, although an application for environmental release may have been received or
- authorisation was granted for environmental release in the past, but that authorisation has expired.

The seed industry has undertaken significant efforts to reduce the incidence of LLPs in seed through adoption of best practice protocols for trait development, breeding, field trials, and seed production and testing to affirm purity of seed (e.g. Excellence Through Stewardship, n.d.). These protocols are more stringent for transgenic seed than those generally employed in conventional breeding and include isolation of plantings, cleaning of machinery and equipment, rogueing, management of pollination, and labelling, inventory and disposal of material. If the seed industry could eliminate LLPs entirely,

it would do so in order to avoid the unproductive costs of LLP situations (SAA, 2009), including those that may occur in the food production system after harvest.

However, even with the implementation of these quality control measures, unintentional mixing of seed cannot always be prevented from occurring in agricultural production systems because of the complexity of modern agriculture. Testing at different points throughout the production system can give conflicting results (due to limits of quantification, sampling errors, etc.), introducing uncertainty as to the effectiveness of best practice protocols and limiting the ability to determine whether there is an LLP in any given seed lot or shipment.

National systems for environmental risk/safety assessment and dealing with LLP situations

Many countries have comprehensive regulatory systems for the assessment of the risk/safety of transgenic plants proposed for environmental release. In any given country, there may be several ministries involved in the evaluation of such plants. Typically agriculture- and environment-based ministries have the primary responsibility for evaluating the consequences of environmental release of transgenic plants.

Addressing an LLP situation nationally may involve more than one or two ministries and can be complex. Usually those ministries responsible for overseeing the evaluation of applications for commercial cultivation (unconfined release) of transgenic plants take a lead role in any environmental risk/safety assessment and may also be involved in the management of LLP situations. Which agencies are involved may depend upon the circumstances of the situation, such as the source of the LLP (commodity or seed) or the particular trait(s) involved. Additional ministries, agencies and government offices may also be involved in addressing a particular situation. These can include quarantine and inspection services, seed quality agencies and plant variety protection agencies, as well as agencies responsible for environmental management and public affairs.

However, even when systems for environmental risk/safety assessment for authorisation for cultivation are in place, some countries' legislative frameworks do not allow for such an assessment in an LLP situation.

While some countries have not experienced LLP situations, several have dealt with at least one incidence of LLP in the context of the environment, either in seed or from certain commodities. Some countries have more experience with LLP situations involving a commodity source than a seed source (see Annex 1.A1 available at OECD, 2013).

Most countries have not to date developed explicit rules or policies to address LLP situations in the environment. However, a few have published policies and guidelines or elaborated more general strategies to limit the occurrence of unauthorised transgenic plants in the environment, including that from LLP situations. These policies and plans serve to communicate to the public the government's approaches to dealing with the potential for environmental risk from LLP situations and to clarify the responsibilities of various stakeholders, including the potential industries involved (e.g. seed production, breeding, trading, transport), in order to limit, as well as prepare for, a potential occurrence of LLP in the environment.

National authorities in the importing country may become aware of (or identify) an LLP situation through a variety of mechanisms, including the following:

1. notification by another country, such as the exporting country, or a regional authority

2. notification by another government authority in the importing country (e.g. seed quality agency)
3. notification by the seed and grain handling industries, including producers, or importers or the owner of the imported plant material or
4. notification resulting from sampling and testing regimes of the government or others.

For national authorities in the importing country, an LLP situation in the environment from seed or commodities may represent a risk to the environment that may require environmental risk management. In addition, an LLP situation in the environment presents a situation of regulatory non-compliance with legislative requirements of the importing country where the plants are not authorised for cultivation (unconfined release). Regulatory agencies may be required to take action to address an LLP situation. In such cases, an environmental risk/safety assessment can support activities to: 1) manage any risks to the environment in a manner commensurate with the risk presented; and 2) achieve compliance with national legislative frameworks. Generally, the primary purpose of an environmental risk/safety assessment in an LLP situation is to characterise the situation and the risk that may be present and to inform environmental risk management. However, the information developed for the environmental risk/safety assessment may also be useful in managing the situation for achieving compliance. The response by an importing country to an LLP situation can vary, depending upon the situation itself and the legal framework.

Principles for determining environmental risk/safety for transgenic plants

The goal of the environmental risk/safety assessment is the same for authorisations for commercial cultivation (unconfined release) as it is in LLP situations: to determine the environmental risk/safety. The general principles for determining environmental risk/safety are the same for an LLP situation in the environment as they are for an authorisation of a transgenic plant for unconfined release. These are stated in the OECD *Safety Considerations for Biotechnology: Scale-up of Crop Plants* (OECD, 1993a). The "scale-up" document describes risk analysis[5] as being: "based on the characteristics of the organism, the introduced trait, the environment into which the organism is introduced, the interactions between these, and the intended application". The:

> … knowledge of and experience with any or all of these provides familiarity which plays an important role in risk/safety analysis […] Familiarity is not synonymous with safety; rather, it means having enough information to be able to judge the safety of the introduction or to indicate ways of handling the risks. A relatively low degree of familiarity may be compensated for by appropriate management practices. Familiarity can be increased as a result of a [field] trial or experiment. This increased familiarity can then form a basis for future risk/safety analysis. (OECD, 1993a: 8).

Further, "familiarity comes from the knowledge and experience available. Familiarity with the crop plant, environment, trait and interactions facilitates a risk/safety analysis" (OECD, 1993a: 29).

In developing an approach to environmental risk/safety assessment of recombinant DNA organisms, the OECD made recommendations in *Environmental Safety Considerations* that were further elaborated in the "Scale-up of crop plants" document. These have been accepted as operational principles worldwide, that countries:

- "use the existing 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 applications in agriculture and the environment by means of an independent review of potential risks on a case-by-case basis[6]
- conduct the development of recombinant DNA organisms for agricultural or 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".

Normally, environmental risk/safety assessment is carried out prior to release into the environment. The above principles apply to evaluation of the stepwise development of an organism for its intended use and this development is based upon data and information gathered until an appropriate amount is consolidated in order to do an environmental risk/safety assessment for commercial cultivation (unconfined release). Usually assessments are done case-by-case and knowledge derived from one environmental risk/safety assessment can be applied to subsequent assessments. The stepwise development of a transgenic organism allows the identification of information and the accumulation of data that supports the environmental risk/safety assessment of the organism for uses at a broader scale. Even though in an LLP situation it is likely that the unauthorised plant has already been found in the environment or environmental release may be imminent, the principles indicated above still apply because:

1. available knowledge and experience can guide the environmental risk assessment
2. an environmental risk/safety assessment can be used to evaluate the potential for risks to the environment in a particular LLP situation
3. stepwise development of the transgenic plant may or may not be underway in the importing country, but use of information and understanding from previous assessments of the same or similar plant domestically, regionally and from other countries may inform the assessment. (OECD, 1986)

Based upon these principles, an environmental risk/safety assessment may be undertaken to identify and evaluate any risk presented by an LLP situation in seed or from certain commodities and the existing familiarity with the components of the situation can provide a basis for such an assessment. The resulting assessment can also inform what actions may be necessary to achieve adequate management of any scientifically identified environmental risk presented, e.g. through standard agricultural practices, the need for additional measures, etc. In addition, such actions may also be useful in bringing the situation back into regulatory compliance. These topics are discussed below in the section on the "Use of information".

Table 1.1 summarises similarities and differences of a risk/safety assessment undertaken in response to applications for authorising unconfined environmental release of a transgenic crop versus an LLP situation of an unauthorised transgenic plant.

Table 1.1. **Environmental risk/safety assessment in low-level presence situations: Key similarities and differences with applications for commercial release**

Type of assessment	Application for authorisation	Low-level presence situation
Purpose/focus of assessment	Determine environmental risk/safety of proposed unconfined (commercial) environmental release of a transgenic plant	– Characterise LLP situation (what, where, when, how) – Determine environmental risk/safety of the unauthorised transgenic plant and the LLP situation
Release to the environment intended	Yes	No
Timeframe for assessment	Defined period for assessment	As soon as possible
Sources of information	Detailed information provided directly to regulator by applicant	Information may be obtained by the regulator from various sources (e.g. application under evaluation, developer, other relevant regulatory assessments, published sources)
Information considered	Biology of the crop plant, transgenic trait, environment and interactions, familiarity with cultivation of same or similar transgenic plants	Biology of the crop plant, transgenic trait, environment and interactions, familiarity with cultivation of same or similar transgenic plants
Identity, amount and location of transgenic plants	– Known – Defined in application	– Knowledge may be incomplete – May be determined in environmental risk/safety assessment
Regulatory action	Decision on authorisation of release to the environment	– Return situation to compliance – Manage risks

Availability of data and information to perform an environmental risk/safety assessment in an low-level presence situation

LLP situations in the environment are dynamic, and a relatively rapid assessment of the risk of the situation is needed for appropriate action to occur in a timely manner. For example, a seed lot containing an LLP of unauthorised transgenic seeds may have already been planted upon discovery of the LLP situation or unauthorised transgenic plants may have been found along a transport route as a result of commodity spillage and subsequent germination. An environmental risk/safety assessment of the LLP situation is usually needed within weeks, days or even hours rather than within the measured pace a national authority might in general apply to an application submitted for authorisation within the existing legal structure. In LLP situations, national authorities face numerous challenges, including that relevant data for the environmental risk/safety assessment may be lacking because it is either unavailable or inadequate or it may not be possible to request additional needed data from an "applicant" through formal procedure, as in an authorisation process. Given the need for a relatively rapid environmental risk/safety assessment in an LLP situation, a profile of the environmental risk presented, the availability of relevant data and information can affect the speed at which a risk assessor can make an assessment of any potential for risk to the environment.

Data and information regarding relevant characteristics of the transgenic plant involved in the LLP situation in the environment, the behaviour of the plant in the environment, including agricultural practices, the LLP situation and the trait may be available from a variety of sources, most likely including the following:

1. domestic authorisations of the particular transgenic plant imported for food and feed
2. application(s) submitted for the particular transgenic plant (review not completed by the importing country)

3. authorisation for commercial cultivation (unconfined release) for the particular transgenic plant from the authorising/exporting country
4. food, feed and environmental authorisations of the transgenic plant by the exporting country
5. authorisations[7] completed for commercial cultivation (unconfined release) as well as completed environmental risk assessments of the same or similar transgenic plant in countries other than the exporting country
6. domestic authorisations for commercial cultivation (unconfined release) of similar transgenic plants (traits, genes, constructs) in the environment
7. data and information from the developer, producer, farmers and other involved industries
8. OECD trait, plant biology and evaluation documents (see OECD BioTrack Website)[2]
9. publically available databases (domestic/international)
10. peer reviewed published literature
11. direct communication with authorities in other countries, particularly the exporting country or authorising country
12. detection procedures suitable for the LLP situation
13. information and experience with similar LLP situations (see also Annex 1.A1 in OECD, 2013).

Given the criticality of an efficient and effective environmental risk/safety assessment in response to an LLP situation, use of already existing domestic and/or internationally available knowledge and experience can give the risk assessor a "head start" in terms of performing the assessment, saving valuable time. As indicated above, in the case of asynchronous authorisation, two obvious sources of information are: 1) that developed for assessment in the country in which the transgenic plant was authorised; and 2) that submitted to regulators for assessment in the importing country. However, the risk assessor may need to actively and rapidly access information from a wide range of sources to obtain sufficient information to make an assessment of the risk/safety.

Access to information can be facilitated by the use of websites containing databases that list authorisations from domestic, regional and sources from other countries. At a minimum, in the type of LLP situation that is being discussed here, the transgenic plant involved in the LLP situation would previously have been evaluated and authorised in another country or several other countries. Entries into these databases may include detailed environmental risk/safety assessments or provide valuable direction as to where this information may be found.

Such existing databases currently include:

- BioTrack Product Database[8] hosted by the OECD
- Biosafety Clearing-House[9] under the Cartagena Protocol on Biosafety
- Crop Database hosted by the Center for Environmental Risk Assessment (CERA, 2012)

- GM Approval Database[10] hosted by the International Service for the Acquisition of Agri-Biotech Applications
- national and regional biosafety websites.[11,12]

Knowledge of the unique identifier designed for individual transgenic plants based on transformation events and authorised by national authorities can serve as a key to facilitate access to information from these databases (OECD, 2006b).

To enhance information sharing between countries, the OECD BioTrack website provides information on biotechnology regulatory contacts for OECD and participating countries, including information on regulatory frameworks and access to OECD biology and trait documents. The Biosafety Clearing-House (BCH) contains information on both the regulatory frameworks of the participating countries and on the available guidance for environmental risk/safety assessment as well as the results of environmental risk/safety assessments for specific transgenic plants conducted according to a specific legislative framework. Direct communication with regulators in other countries as well as using information on the environmental risk/safety assessments done in those countries can facilitate risk/safety assessment in an LLP situation. It is important for countries to keep their information current in these databases to maximise their usefulness.

Information may be accessible directly from the authorities in the authorising country (or countries) and from scientific literature. Collaborative working relationships between national authorities in different countries and/or with industry and public institutions have enhanced access to information, and establishing ongoing communication may be beneficial to this process. The importance of working relationships between national authorities cannot be over-emphasised. For conducting environmental risk/safety assessment of an LLP situation, only limited information may be immediately available to the importing country. It is sometimes difficult for importing countries to obtain the information needed from the developers and/or companies involved, particularly when the scale of production is not large. In such cases, the responsible government agency in the exporting country may provide information that can be shared with the importing country. When the LLP situation results from asynchrony of authorisation such that the transgenic plant involved is authorised in the exporting or other countries, a great amount of data will have accumulated.

Information may be available on the trait or phenotype within the crop plant in the particular environment and/or in a variety of environments along with the identification of any unintended effects in the environments of countries in which authorisations have been made. Such information may be adequate for the purpose of an environmental risk/safety assessment of the LLP situation, depending upon the specific regulatory requirements of the country or region.

Characterisation of the introduced trait may come from an authorisation or application received for food and feed, and/or environmental release of the same or a similar plant. Many times the information on molecular characterisation of the introduced trait is very similar for these types of authorisations (OECD, 2010). Further, a feed or food safety assessment (e.g. done according to the Codex Alimentarius Commission [2003]) contains information that may be useful in an environmental risk/safety assessment including a description of the transgenic plant, the unmodified plant, the donor organism of the introduced genetic material and a characterisation of the genetic modification. While information developed for food or feed safety assessments may be limited for the

performance of an environmental risk/safety assessment (e.g. compositional analysis), it may set the context for an assessment of an LLP situation in the environment.

In cases where applications for a review necessary for an authorisation for commercial cultivation (unconfined release) have not been received and more data or information are needed to address the LLP situation, the data and information available from the additional sources mentioned above may be assessed for adequacy for the purpose of an environmental risk/safety assessment in the importing country. In addition, communication between the importing and exporting countries can facilitate the exchange of as much data and information as possible within the boundaries of legal constraints. The importing country may also work with the developer of the unauthorised transgenic plant to obtain as much relevant data and information as possible to address the LLP situation efficiently.

Environmental risk/safety assessment in an LLP situation

When approving a transgenic plant for potential cultivation, usually the environmental risk/safety assessment assumes 100% exposure over an extended period of time, i.e. the plant is cultivated on potentially very large areas of land. This is an assessment of a product for intentional use. However, when assessing an LLP situation, the context may be different. The determination of environmental risk that an unauthorised plant may pose is based not only on the hazards identified, but on the potential exposure, which will be related to the scale of an LLP situation. The amount and degree of information needed may be different for an LLP situation because of the reduced scale and the purpose of the assessment. By definition, generally, an LLP situation is at a scale reduced from that assumed present in a risk/safety assessment for authorisation for large-scale cultivation of the same plant. In an LLP situation, the environmental risk/safety assessment is not intended to lead to an authorisation. However, the results of the assessment can be useful in supporting environmental risk management decisions by scientifically evaluating potential options for managing any risk presented.

The purpose of the following discussion of scale, the trait, and the plant and the receiving environment of the importing country is to indicate how the available knowledge and experience, data and information can facilitate a rapid environmental risk/safety assessment of an LLP situation; the timeframe for the assessment and decisions is much shorter than for an authorisation for cultivation. It does not explain explicitly how to do such an assessment. It is noted that the types of information used are generally the same as for the review of an application for authorisation where much of the information is supplied in the application itself. There is ample discussion of these types of information and their importance to environmental risk assessment in previous OECD publications (OECD, 1992; 1993a; 1993b). The basic safety issues that may potentially be of concern were identified in these publications. They include gene transfer, weediness, trait effects, genetic and phenotypic variability, genetic material from pathogens and worker safety.

Scale

Each country makes its own determination of what is considered to be an LLP, most often on a case-by-case basis. In terms of the environmental risk/safety assessment, several approaches to determining the scale (i.e. amount of seed distributed spatially and temporally) of the presence of the unauthorised transgenic plant involved in the LLP situation may be useful. For commercial seed containing an LLP that has been planted,

the scale may be determined through information about the anticipated distribution and period of release, especially information on seed distribution, and this is usually known by seed companies and farmers (e.g. where seed lots have been distributed or fields planted with that seed), although this may not always be the case. Results of *in situ* testing may also be available or useful, depending upon the situation. Availability of detection methodologies may provide information for this purpose. This testing may occur in unplanted seed, in plants in the field or in the harvested commodity and is a means of understanding the potential environmental distribution. Other potentially useful types of information may include an identification of the source of the unauthorised transgenic plant, information on seed lots (whether transgenic or not) that are not expected to contain unauthorised transgenic seed and information on quality control of seed (whether transgenic or not) in seed-producing countries. For both commodities and seed, knowledge of crop plant-specific international movement can provide information to allow examination of the amount and pathway(s) of distribution; such information could include known distribution routes, shipping manifests and trade statistics.

The trait

Molecular characterisation allows for the verification of the trait and genotype, which in turn supports the characterisation of the phenotype. Depending upon the situation, the verification needed may come from data and information about the protein, construct and/or the specific event. The nature of the genetic modification, particularly any protein(s) expressed by the transgenes (OECD, 2010), and biological functionality of the gene products allows for a determination of how similar they are to those found in transgenic plants authorised either domestically, regionally or in other countries. Useful data and information for an assessment of an LLP situation can generally be extrapolated from that of the same or almost the same transgene; regulatory elements; transformation methods; introduction into the same genetic background as approved lines; similar expression levels; relevant field test data; lack of additional unintended genetic material; and effects of expression of a very similar protein.

When the LLP plant is known to be similar to an existing authorised transgenic plant, much of the information from previous domestic, regional or other country determinations becomes relevant and directly applicable. Such knowledge facilitates the identification of potential adverse impacts such as known toxicity of the gene product or effects on non-target organisms (OECD, 1993a). The following questions point an assessor to the types of information that may prove useful in determining the degree of familiarity with the trait in the unauthorised plant in an LLP situation:

- Does the unauthorised plant belong to the same plant species as previously evaluated or authorised transgenic plants?
- Does the unauthorised plant contain the same or similar trait, transgene, genetic components and/or regulatory elements as previously evaluated or authorised plants?
- Was the transformation method the same as that used in a previously evaluated or authorised plant or, if not, does use of a different method present any additional issues?
- Is the same or a very similar protein expressed in a previously evaluated or authorised plant? Are protein expression levels and/or patterns similar to previously evaluated or authorised plants?

- Are field test data available that support the conclusions of other assessments of similar transgenic plants?

While data and information addressing all of the above questions may not be needed to understand the behaviour of the unauthorised plant, previous assessments of the same or a similar plant that have addressed what potential adverse effects might be predicted for the unauthorised plant can contribute to a rapid understanding of whether the LLP situation might result in any adverse effects. More or less information will be needed, depending upon the particular LLP situation, how quickly decisions are needed and the core information and comprehensiveness of that information needed to take those decisions.

Information on the unauthorised plant, when available, can further confirm the applicability of existing general knowledge and/or experience of how the trait can affect the plant, including how it affects growth, survival and reproductive ability. In cases where the unauthorised plant contains combined traits, familiarity with the combination of traits may be useful. Domestic field trial data that may be available can support conclusions regarding the environmental effects of a particular trait in the LLP situation, particularly if an application for authorisation has been received.

The available broad domestic or global experience and knowledge of how the major traits being used today, particularly the herbicide-tolerant and insect-resistant traits, affect different plant types in different environments may provide a range of possibilities of how the trait may affect the behaviour of the plant in the environment of a particular LLP situation. Such information can include that on the same trait introduced into different plant species, and knowledge and experience with similar traits in the same crop plants developed through traditional plant breeding. Since a given trait may perform differently in different plant species, the existing combined global knowledge and experience of a particular trait in these different plant species gives a breadth of understanding that may be useful in determining the potential range of responses of the plant-trait combination in the specific environment of an LLP situation.

There are several examples of genes and traits that have been evaluated by many countries. The phosphinothricin acetyltransferase (PAT, conferring tolerance to glufosinate ammonium), and 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS, conferring tolerance to glyphosate) and *B. thuringiensis* crystal (Cry) proteins in the LLP situations to date have essentially been identical to those in similar authorised plants and have been produced from the same or similar gene constructs. Much information is available on these proteins and their associated expressed traits from the OECD (OECD, 1999a; 1999b; 2007). The herbicide tolerance and insect resistance traits resulting from most of these proteins as well as application of the complementary herbicides – in case of herbicide tolerance – have undergone multiple assessments and environmental reviews (CERA, 2011a, 2011b, 2011c, 2011d; Heard et al., 2005; Marvier et al., 2007; Wolfenbarger et al., 2008; Perez-Jones and Mallory-Smith, 2010; Lang and Otto, 2010; see also national and regional decision documents accessible thorough the OECD BioTrack database) with subsequent global commercialisation over the past 20 years in a variety of crop plants. For example, *cry1ac* has been authorised in three crops containing a variety of constructs in 11 countries, while glyphosate tolerance has been authorised by 13 countries in 8 different crop plants. Thus, the origin, the genes and proteins produced and the functioning in plants of associated genetic regulatory elements and markers (i.e. ampR, NOS, 35S CaMV), together with the respective risk assessments and risk managements, have been well documented in regulatory decision documents globally.

This information may provide a solid knowledge base for the extrapolation of any environmental risk/safety assessment for an LLP situation containing these genes, expressed protein(s) and the resultant trait.

Plant and receiving environment of the importing country

The majority of LLP situations to date have involved "common" crop plant species and trait combinations that have been widely adopted and are under large-scale cultivation where authorised. There is substantial knowledge and experience with these plant species as they are grown regularly within the countries in which LLP situations can occur, as non-transgenic or transgenic crops. Familiarity with the biology of the crop plant and its behaviour in the receiving environment in the context of the existing agricultural practices (cultivation and environmental management) of the country or region can be used to identify aspects of the environment that may potentially be affected in an LLP situation. This can facilitate rapid assessment of the potential for any environmental risk to be presented by an LLP situation in seed. The importing country with the LLP situation may use this understanding to identify the unique or different aspects of its country/region compared to the exporting country (and other countries) where assessments for authorisation of the transgenic plant have already been completed.

The use of existing information on cultivation of a plant species can facilitate rapid performance of an environmental risk/safety assessment and the development of environmental risk management plans in an LLP situation. Agricultural practices (e.g. crop rotation, tillage, planting dates, herbicide use, control of endemic pests and diseases) may vary within the same plant species and between countries or regions because of variations in climate, soil and other factors. However, most crop plants, including transgenic ones, are normally restricted to (or dependent upon) the managed environment due to extensive domestication (this may vary according to the species of plant). Cultivation of authorised transgenic plants may include additional practices beyond standard ones, depending upon the trait(s) and/or any risk presented (e.g. insect resistance management). Practices such as herbicide usage for weed and volunteer control or use of pesticides/fungicides to manage pests and diseases may be important for determining the risk/safety of unauthorised seed containing traits for herbicide tolerance or insect resistance. Much information on different crop plant species and associated agronomic practices in the environment is available in the OECD biology documents[2] as well as from various publications of national authorities. Over 30 OECD biology documents are currently available, including documents for the major commodity crops (corn, cotton, rapeseed and soybean) (OECD, 2003; 2008; 2012; 2000). These OECD documents have proven useful in various national reviews, including those of LLP situations.

Important information for environmental risk/safety assessment in an LLP situation may also include that on any means of spread and persistence of the plant. Many crop plants, including most of the important seed and commodity crops, have lost the weed-related traits of their wild progenitors through domestication and this history may be well understood. The OECD biology consensus documents (see above) may provide baseline information on the ability of a plant species to spread and persist in the environment.

However, some crop plants outcross prolifically within the crop or to sexually compatible species. When such plants are cultivated in areas geographically close to populations of sexually compatible wild or weedy relatives, the potential for exchange of

genetic information may be a consideration in an environmental risk/safety assessment. Where populations of wild or weedy relatives occur, information is generally specific to a country or region: the OECD biology consensus documents may provide baseline information on several plant species' feral, wild and weedy relatives and the distribution of these relatives.

Some plant species only exchange genetic information at very low rates. In general, a reduced ability of a plant to cross with other plants of the same species or wild or weedy relatives would limit the possible extent of outcrossing, and thus reduce concern that the transgene may have moved, through the exchange of genetic information, from the original area of release.

Thus, the potential of the introduced transgenic trait to spread in the environment through the exchange of genetic information between the unauthorised transgenic plant and associated crop plantings or wild or weedy relatives may be of particular interest in an environmental risk/safety assessment. Once identified, these factors can be evaluated in the context of the existing agricultural practices for either the unmodified plant or similar authorised versions of the transgenic plant and provide relevant information in evaluating an LLP situation.

Currently, many traits are introduced into crop plants to directly affect target pest species and disease organisms. In addition to the target species, a variety of organisms interact with crop plants in the field. The potential for direct or indirect effects on beneficial or endangered organisms depends upon this interaction and is directly dependent upon scale. The nature of the trait (e.g. virus resistance or insect resistance) may indicate whether a safety issue is of concern. Familiarity with the fauna of a region will indicate whether a trait in a particular crop plant is of concern. Information from previous authorisations may provide this knowledge efficiently, including whether standard agricultural practices provide sufficient management of the concern.

When commodities approved for food and feed use alone are the source of an LLP situation found in seed to be planted or in fields already planted, existing familiarity with the cultivated plant as indicated above, can be used for the environmental risk/safety assessment. However, additional information, such as that on the maintenance of the transport route, including weed management, may be useful when such plants show up growing outside of cultivation, e.g. in environmentally disturbed areas such as roadsides and railroad track beds. Knowledge of the conditions for plant growth and survival in disturbed environmental settings, generally sub-optimal for many domesticated plant species as they require human maintenance, and whether the plant can just survive or can form self-sustaining populations and become weedy can inform an assessment of the potential for persistence or spread of the plant and/or trait. Knowledge of the presence of wild and weedy relatives in the local area or nearby compatible crop plantings can also be a factor in determining the potential for spread and/or persistence along with whether the plant species is listed as a weed in the region. Even if the plant species is not cultivated within the country, information is available from the OECD about the commonly traded crop plants.

Risk profile

When an environmental risk/safety assessment of an LLP situation is undertaken, the goal is to determine any risk presented and to scientifically evaluate potential options for managing any risk presented. In a relatively short period of time, the identity of the unauthorised plant material may need to be confirmed, the potential for adverse effects be

determined and actions taken to minimise any identified risk presented by the LLP situation. A risk profile characterising the situation may be rapidly assembled based upon data and information from reviews of the same or similar authorised plants and/or existing knowledge and familiarity with the plant, trait, environment and their interaction. The risk profile recognises the scale of the LLP situation and may expeditiously inform decisions to manage or mitigate any risk presented as well as to return the situation to regulatory compliance.

The following process can be used to develop a risk profile to expeditiously address an LLP situation in the environment of the importing country subsequent to identification of the presence of an unauthorised transgenic plant:

- determine where the LLP situation has been found in the environment and the potential distribution of the unauthorised transgenic plant
- identify relevant sources of information, including previous assessments of that unauthorised transgenic plant available either domestically, regionally or from other countries
- determine if those assessments identified any potential hazards and whether/how these relate to the importing country's protection goals and could potentially affect the receiving environment harbouring the unauthorised transgenic plant
- determine/consider whether there are pathways for distribution of the unauthorised transgenic plant in the LLP situation through which the identified hazard can cause adverse effects in the receiving environment
- assess the likelihood and consequence of those adverse effects being realised.

The trait likely to be expressed and any similarity of such trait expressed in previously authorised plants provide an invaluable starting point. Further data and information may or may not be needed depending on how familiar the risk assessor is with the plant species and the trait in the environment in question. If the plant species is well understood, as in most LLP situations with seed, then the focus of the assessment is on the trait(s) in the unauthorised transgenic plants and any risk of harm it might present to the environment when present at low levels. The resulting environmental risk/safety assessment can characterise the risk that may occur or has occurred given the specifics of the LLP situation (case-by-case). The assessment may include an evaluation of the management options to address any risk to the environment that might be presented, such as an evaluation of existing or modified distribution systems and agricultural practices used with the particular plant species. The assessment can also provide the needed scientific basis to inform broader management objectives, such as those to return the situation to compliance with regulatory requirements. In the context of this discussion, such management options may include mitigation of any further release of unauthorised plants into the environment and/or remediation of any release that has already occurred.

If the unauthorised LLP plant is similar to existing transgenic plants authorised domestically, regionally or in other countries, much of the information from those previous assessments and conclusions of safety may be directly applicable. Table 1.2 provides several examples of the knowledge and information that may exist, depending upon the case. Any aspects of the receiving environment such as cultivation practices, biological aspects such as those for potential dissemination, persistence through natural means (e.g. pollination, dormancy, volunteers, etc.) or the potential for negative effects on beneficial or endangered species can be examined, as can human factors such as

transport, handling, spillage, planting. Depending upon the trait, these elements can be used to determine the applicability of the results of previous assessments in making a prediction of the risk presented by the LLP situation. In this context, knowledge of the source(s) and the scale of the LLP situation spatially (e.g. area and/or location) and temporally and of the amount of the unauthorised transgenic plant involved (e.g. a limited amount of seed might be distributed/spread over a wide geographic area) is relevant to the assessment. Should the trait influence the plant's ability to persist and spread, an assessment may evaluate whether the change in behaviour could lead to an adverse consequence. In addition, the assessment may evaluate whether such changes could present additional or novel pathways to harm within the environment of release. Finally, an assessment may evaluate whether such changes might directly impact the ability to control or manage the situation using existing practices.

In performing such an evaluation, an assessor's knowledge of a particular managed agricultural environment, including information on the surrounding partially managed or natural environment, would inform the determination of risk/safety. At the conclusion of the assessment, any differences in risk profile compared to previously authorised and/or similar plants can be determined including whether a different adverse consequence has been identified or whether there is a difference in the unauthorised plant's behaviour in the environment.

The environmental risk/safety assessment may identify areas of uncertainty that may need to be addressed by additional information. This may depend upon how familiar the risk assessor is with the plant and the trait in the environment in question. More data or information may be necessary, such as for molecular characterisation or on the potential of the trait to increase weediness in a particular plant.

Ultimately, the environmental risk/safety assessment takes into account agricultural practices and the effectiveness of these practices to manage any risk presented either through limiting or removing the unauthorised plant from the environment. Familiarity with agricultural practice can indicate:

- where risk management can adequately be applied using standard agricultural practices or
- when additional remedial or mitigating measures are needed.

Familiarity with agricultural practice may also potentially inform any actions to bring the LLP situation back into compliance with regulatory requirements.

To date, the LLP situations in the environment from seed and commodities that can function biologically as seed have allowed for relatively straight-forward, case-by-case, comparative, scientific assessments of risk/safety based for the most part on existing information. As a result, when assessments have been carried out, it has been determined that the low-level presence of these unauthorised transgenic plants in seed or commodities in the environment posed a low level of risk, given the impacts and scale of the situations.

This conclusion was based on the review of available scientific data, the limited amount of the unauthorised plant in the environment, and comparison with either the unmodified plant or the close similarity of the unauthorised plant to authorised transgenic plants which had cleared regulatory review in the importing country.

Table 1.2. **Potential example scenarios indicating types of existing knowledge and information that may be used by an importing country to facilitate an environmental risk/safety assessment of an LLP situation**

Scenario 1: Protein/construct/event authorised for import (food, feed and processing), but not for cultivation	Scenario 2: Protein/construct/event not authorised in the importing country but the inserted gene and protein produced are the same as or very similar to other transgenic plants authorised in the importing country	Scenario 3: Protein/construct/event that has no authorisation in the importing country
Characterisation of the introduced trait completed in the importing country	Characterisation of the same or similar gene and protein in the importing country Characterisation of the introduced trait completed in the exporting or authorising country	Characterisation of the introduced trait completed in the exporting or authorising country
Agricultural areas where the crop is grown provided by seed company/industry	Agricultural areas where the crop is grown provided by seed company/industry	Agricultural areas where the seed crop is grown provided by seed company/industry
Experience with cultivating this crop in the importing country. Specifically, the focus would be on the crop's inherent properties related to weediness (persistence and invasiveness) and pest management, depending upon the trait	Experience with cultivating this crop in the importing country	Experience with cultivating the crop in the importing country, particularly regarding the tendency to persist or spread in the environment Experience with the trait (or similar traits) in the other crops
Agricultural practices, with a special emphasis on those associated with the trait, such as the use of the target herbicide in the case of an herbicide-tolerant trait	Existing environmental risk/safety assessment data and experience with the unauthorised transgenic plant/event line within the importing country for the gene and expressed protein. The environmental risk/safety assessment of the same or similar authorised transgenic plant line or event in the respective cropping system, focusing on the likelihood that the trait would alter the crop's weediness or have an effect on non-target organisms	Environmental risk/safety assessments available from other countries
Environmental risk/safety assessments available from other countries	Environmental risk/safety assessments available from other countries	Information about the receiving environment and common agricultural practices in that receiving environment
Experience and information from similar crop/trait combinations and deemed relevant by the importing country	Experience and information from similar plant/trait combinations deemed relevant by the importing country	Other considerations, such as the level of exposure to beneficial organisms, humans and the environment
Relevant OECD consensus documents	Relevant OECD consensus documents	Relevant OECD consensus documents

Note: In these cases, the crop plant is grown in the importing country (unmodified or similar traits in authorised transgenic plants).

In instances where an importing country has not carried out a previous assessment of the same or similar plant, globally available information may be used: the biology of the plant, the trait, and the interaction of these in the receiving environment. Thus, there is much information about these factors that may be useful to expedite an environmental risk assessment in an LLP situation to inform the appropriate action needed to protect the specific environment in the importing country. International databases can function as a source of information to evaluate the adequacy of available risk/safety information for the requirements of a particular legislative framework (see above). The aggregate of this broader set of information can give the assessor an indication of the range of potential interactions with the environment of the trait in the same plant species and in other species and this may be directly applicable to the environment of the importing country.

This has been especially true with the LLP situations to date in which many countries have evaluated the crop plant with the herbicide-tolerant and insect resistance traits grown and traded globally. However, when an application for authorisation has already been received by the importing country, much of the needed information may already be available.

Use of information and an environmental risk/safety assessment for management of low-level presence situations in seed

Possible approaches to the management of low-level presence situations in seed

The "scale-up" document (OECD, 1993a) describes environmental risk management as "the way appropriate methods are applied in order to minimise scientifically identified risks … In principle, appropriate management is based on and should be in proportion to the results of the risk/safety" assessment. "Risk management encompasses all aspects of the management of the organism indirectly through management of the environment into which the organism is introduced, or directly, by management of the organism itself."

In general, management of an LLP situation may focus on the goals of protection of the environment (environmental risk management) and/or returning the situation to compliance with the requirements of a country's legislative framework. An environmental risk/safety assessment may be useful in informing decisions for environmental risk management and returning the LLP situation to compliance with the regulatory requirements of the country or region, recognising that the use of an environmental risk/safety assessment for this purpose may depend on the provisions of the legislative framework of the country. An environmental risk/safety assessment may not be needed to address a particular LLP situation due to the adoption of processes to handle LLPs or, in contrast, the framework may not allow management measures for LLP situations in general to be based upon the results of an environmental risk/safety assessment. When performed, an environmental risk/safety assessment can be used to characterise the situation, including identifying any risk associated with the situation and identifying the measures either in place or needed to manage any risk presented. Overall, management measures undertaken by a country will likely address environmental risk management as well as measures to return the situation to compliance. The information provided by the risk assessment can identify whether risk management of the situation is inherent in the agricultural management practices already at hand; whether additional measures for mitigation are needed and, additionally, whether these same measures will be useful in returning the situation to compliance.

Familiarity with the biology of the crop plant and the associated agricultural practices can not only facilitate rapid assessment of any risk presented by an LLP situation in seed, but the ramifications for mitigation or risk management of the situation. In the LLP situations to date, major crop plants involved were corn, cotton, rapeseed/canola and soybean with commonly inserted genes for insect resistance and herbicide tolerance. Environmental risk/safety assessments have been useful in informing decisions for managing these situations, particularly in limiting or mitigating the spread and persistence of the unauthorised plant. Knowing the source of the LLP in seed may facilitate limiting further introduction of the LLP seed into the environment given the distribution of the plant, the ability of the plant to establish and spread, and the methods available for control or eradication. However, it may not always be possible to determine whether the source of the LLP of unauthorised plant found in the environment originated in seed or from some other source, such as commodity spillage. In any case, an environmental risk/safety

assessment can identify and evaluate any risks associated with an LLP situation and, depending on the country's legislative framework, provide options for environmental risk management in a manner proportional to any risk presented to achieve protection goals (OECD, 1993a; see also above). The concept of risk management being proportional to the level of risk is standard for all risk assessments. In addition, the same measures may contribute to returning the situation to compliance with legislative mandates; e.g. remediation and mitigation options that ultimately lead to limitation of the maintenance and/or spread and/or removal of the unauthorised plant from the environment and ultimately the seed supply.

The circumstances and timeframe of an LLP situation in seed are other major factors in environmental risk assessment and risk management (e.g. has the seed been planted; if the commodity has spilled, is the season right for germination; if germination has occurred, what developmental stage are the plants at, especially with respect to sexual reproduction – flowering, seed set, harvest?). All of these factors can be time critical for determining the appropriate environmental risk management/mitigation measures, depending upon the risk presented – e.g. removal or destruction of the unauthorised transgenic plants prior to flowering may or may not be important in limiting potential spread or persistence.

A significant factor for food and feed crop plants involved in an LLP situation is whether a food and feed safety evaluation has been undertaken or authorisation given, either domestically or by another country. It may be relevant to consider information from safety assessments of food, feed and processing of the implicated transgenic plant that may exist from different sources, including national sources, in setting the context for an assessment of an LLP situation in the environment. Food and feed safety evaluations can provide relevant information regarding the potential for adverse environmental consequences to wild animals that may inadvertently consume the plant.

If the environmental risk is determined to be insignificant in comparison with the unmodified counterpart or a similar authorised transgenic plant, and if the country's regulatory framework allows for it, one option might be "no action" to remediate or mitigate the particular situation from an environmental risk perspective. Depending upon the situation, seed and/or plants may be limited or removed from the agricultural production system including in the following manners:

- recall of unplanted seed from distributers
- destruction of planted seed once germinated
- allowing planting and/or harvest, but controlling the distribution of any seed or harvested crop produced
- permitting seed already planted to be utilised in a manner where processing procedures devitalize the plant so there is no further potential for plant growth (e.g. biogas utilisation).

Each country will consider appropriate management strategies under its legislative framework, and therefore some of these options may not be feasible.

Although the conclusion of the environmental risk/safety assessment may suggest options that allow the management or mitigation of any risk of the unauthorised plant in a manner commensurate with the level of risk presented, other factors also play a role in determining appropriate management of an LLP situation. An LLP situation is, almost by definition, a situation of non-compliance with regulatory requirements, and in many

jurisdictions there are legal requirements for compliance that also set the context for management for risk. In addition, the complexity of the response may be influenced by, for example, socio-economic factors, legislative mandates, stakeholder preferences or the availability of resources. In addition, the preferences of the grower, seed supplier or industry may also play a role and there are several examples of growers, developers and seed suppliers taking more rigorous action than mandated by the national authority. In many LLP situations, national authorities have demanded destruction, devitalisation or reshipment of seed lots to achieve compliance. However, economic consequences to the farmer, importer and government may also play a role. The responsiveness and collaboration of the industries involved have been critical to addressing past LLP situations. Nonetheless, when performed, the environmental risk/safety assessment itself becomes an overriding consideration in the development of plans to mitigate and manage an LLP situation proportional to the risks presented.

In summary, important environmental risk assessment factors that are considered in developing management plans include:

- the present circumstance of the LLP situation in the seed or commodity, including where the unauthorised plant was discovered
- conclusions of an environmental risk/safety assessment.

Potential ways to proactively address environmental risk for low-level presence situations

In recognition of the fact that LLP situations are anticipated to increase and have the potential to be disruptive to trade and create economic hardship on seed producers, importers, shippers and farmers as attested in responses to the OECD questionnaire (Annex 1.A1 available at OECD, 2013), countries and regions have taken several steps to limit the potential for uncertainty regarding environmental risk. Some authorities undertake environmental assessment of transgenic plants authorised for use as food or feed and for processing in recognition of the potential of these commodities authorised for import to be found in the environment. Thus, when LLP situations in the environment have occurred with such plants, countries have been able to rely on the determination that the risk presented is no greater than that presented in the unmodified plant. This applies to those situations in which the unauthorised transgenic plant is found in planted fields as well as along transport routes due to spillage during commodity transport. Other countries perform assessments for authorisation of commercial cultivation (unconfined release) of the plants that are destined to be imported for only food, feed and processing. When these plants have later been found in the environment, they have not been deemed illegal. In neither of these approaches does it mean that it is acceptable to allow commingling of seed material in an ongoing manner. But, in some situations with identified low levels of an unauthorised plant, there may not be a general concern raised.

Some importing countries have set up comprehensive systems for working with potentially affected domestic government agencies and stakeholders, particularly affected industries, to prevent the import of seed or commodities containing unauthorised plants. Some countries work with the seed and plant-breeding industries to ensure appropriate quality control systems are in place to prevent unauthorised plant material from getting into breeding material. The industries themselves have also incorporated protocols to reduce the prospect of having seed or commodities rejected or destroyed upon arrival in the importing country due to the presence of a low level of an unauthorised plant.

Preventive measures taken by industries are critical to reducing the occurrence of LLP situations.

Preparations for a possible LLP situation have occurred in some countries through the development of communication plans with other national government agencies and through educating stakeholders as to their roles and responsibilities in both preventing and managing an LLP situation. Such close relationships can enable importing countries to address LLP situations in an effective and efficient way.

Several countries, recognising the potential for LLP to occur in seed, have set thresholds allowing for LLP if a food safety authorisation has been done according to the Codex Alimentarius plant guideline either regionally or in a country with a similar food safety review system as the importing country. Since it may be impossible to entirely eliminate LLP in seed, in some cases thresholds have been set to assure an acceptable and predictable supply of seeds. This has been in response to several instances where the LLP was detected at such a low level that it was technically below the level of quantification using validated protocols for testing. In these situations, testing at different stages in the seed distribution system led to conflicting results regarding the presence of LLP in seed. Recognising the inability to entirely eliminate LLP, thresholds have also been adopted by some importing countries to avoid the reduced availability of seeds in cases where it was known that the unauthorised plant had been authorised at least in one other country.

Notes

1. The OECD has described a transgenic plant as a plant with a gene or a genetic construct introduced by a molecular technique (OECD, 1993a: 33).
2. See: *www.oecd.org/biotrack*.
3. This chapter discusses risk/safety analysis as being comprised of "hazard identification and, if a hazard has been identified; risk assessment" (OECD, 1993a). Currently, the term "risk assessment" has replaced the term "risk analysis" as the term most commonly used to indicate both hazard identification and risk assessment.
4. There may be other factors outside the scope of this chapter that affect whether a plant is ultimately cultivated. For example, the importing country may have performed a risk/safety assessment on the plant for food and/or feed use and/or environmental release and concluded the material could be authorised. However, other legal constraints may exist (e.g. government seed variety certification/ registration requirements) so that the plant would not be fully authorised for commercialisation unless these other legal requirements are met. Seed certification or registration is not a component of environmental risk/safety assessment.
5. This chapter discusses risk/safety analysis as being comprised of "hazard identification and, if a hazard has been identified, risk assessment." Currently, the term "risk assessment" has replaced the term "risk analysis" as the term most commonly used to indicate both hazard identification and risk assessment.
6. Case-by-case means an individual review of a proposal against assessment criteria which are relevant to the particular proposal; this is not intended to imply that every case will require review by a national or other authority since various classes of proposals may be excluded.
7. Assessments that did not lead to authorisation, either domestic, regional or from other countries, may also provide useful information. However, there are a variety of reasons an application may not lead to authorisation.
8. OECD BioTrack Product Database available at *http://www2.oecd.org/biotech/*.
9. Biosafety Clearing House of the CBD available at *http://bch.cbd.int*.
10. ISAAA's GM Approval Database, available at: *www.isaaa.org/gmapprovaldatabase.*
11. Biotechnology Regulatory Contacts in OECD Member Countries, available at: *www.oecd.org/chemicalsafety/biotrack/biotechnologyregulatorycontactsinoecdmembercountries.htm*.
12. Search for National Contact (at BCH website), *http://bch.cbd.int/database/contacts.*

Annex 1.A1: Questionnaire on LLP situations and country responses

A questionnaire was circulated in 2009 to gather information on participating countries and observers' experience with low-level presence situations in seed and certain commodities. The comprehensive text of the questionnaire was issued in the original document, in Annex 1 – Annotated Questionnaire for LLP in Seed and Commodities in the Context of Environmental Safety (OECD, 2013).

Responses were collated in Annex 2 of the original document (OECD, 2013). Annex 2 comprised inputs from 19 countries and one observer, namely Argentina, Australia, Belgium, Brazil, Canada, Chile, Czech Republic, Estonia, Japan, Korea, Mexico, the Netherlands, New Zealand, Norway, Philippines, Spain, Turkey, the United States, and the Business and Industry Advisory Committee to the OECD (BIAC).

Most of the information was provided by national authorities during the year 2010, or shortly after. Because more experience is gained to date, and elements of the responses would have probably evolved significantly since the original issue of the document, Annex 2 is not reproduced here to avoid mis-interpretation. However, the original document is available on the OECD BioTrack Website and Annex 2 should be understood as an incomplete source of information that was valid at the time of the circulation of the questionnaire.

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Part II:

Biology of crops

Chapter 2.

Sugarcane (*Saccharum* spp.)

*This chapter deals with the biology of sugarcane (*Saccharum *spp.). It contains information for use during the risk/safety regulatory assessment of genetically engineered varieties intended to be grown in the environment (biosafety). It provides elements on commercial uses of the crop for producing sugar and other products, and on sugarcane payment schemes. It includes information on taxonomy; centre of origin; domestication and cultivation practices; morphological characteristics; reproductive biology; pollination and vegetative growth; genetics; abiotic interactions with nutrients, temperature, water and other stresses; interactions with weeds, pests and pathogens; hybridisation; and health considerations.*

This chapter was prepared by the OECD Working Group on the Harmonisation of Regulatory Oversight in Biotechnology, with Australia as the lead country. It was initially issued in November 2013. Updates have been made on production data from FAOSTAT, in the sub-section entitled "Scale of cultivation", on genome sequencing developments, and relating to recent approvals of genetically engineered sugarcane varieties developed for drought tolerance.

Introduction and uses

Sugar is commercially produced from either sugar beet (*Beta vulgaris*) or sugarcane (*Saccharum* spp.). Sugarcane is a tall-growing monocotyledonous crop that is cultivated in the tropical and subtropical regions of the world, primarily for its ability to store high concentrations of sucrose, or sugar, in the stem. Modern sugarcane cultivars that are cultivated for sugar production are founded on interspecific hybrids between *Saccharum spontaneum* and *S. officinarum* (*Saccharum* spp.) that were then subjected to repeated backcrosses to *S. officinarum*. Commercial varieties in use today are typically generated by crosses between other commercial or pre-commercial hybrids. Sugarcane is an ancient crop and its use as a garden crop dates back to around 2500 BC. The centres of origin for the ancestral species giving rise to sugarcane are thought to be Papua New Guinea, the People's Republic of China (hereafter "China") and India. At present, sugarcane is grown as a commercial crop primarily in South America (e.g. Argentina, Brazil and Colombia), North/Central America (e.g. Guatemala, Mexico and the United States), Asia (e.g. China, India and Thailand), Africa (e.g. Egypt, Kenya and South Africa), Australia and the Pacific Islands. Cultivation practices vary throughout the world, but this chapter aims to outline the main features of sugarcane cultivation. Sugarcane in this chapter refers to the *Saccharum* spp. hybrids as described above. The information presented is that which is available for each country after a comprehensive literature review.

Commercial uses

Sugarcane is grown for its sucrose content and is mostly consumed as refined sugar or other processed products (see below). Raw sugarcane can be squeezed or chewed to extract the juice, which is known as "*caldo de cana*" or "*garapa*" in Brazil, "*chediraz*" in northern India and "*aseer asab*" in Egypt. In some countries in which sugarcane is grown, it is bottled for local distribution or sold fresh from juice bars, cafes and restaurants.

Outside of commercial processing, artisanal processing of sugarcane occurs where sugarcane juice is boiled and cooled to make cakes of unrefined brown sugar, known as "*jaggery*", "*gur*" and "*khandsari*" in India; "*rapadura*" in Brazil; and "*panela*" in Colombia. In India it is estimated that 16.5 million tonnes (t) of sugar are produced compared with 10 million t of these traditional sweeteners (Kansal, 1998).

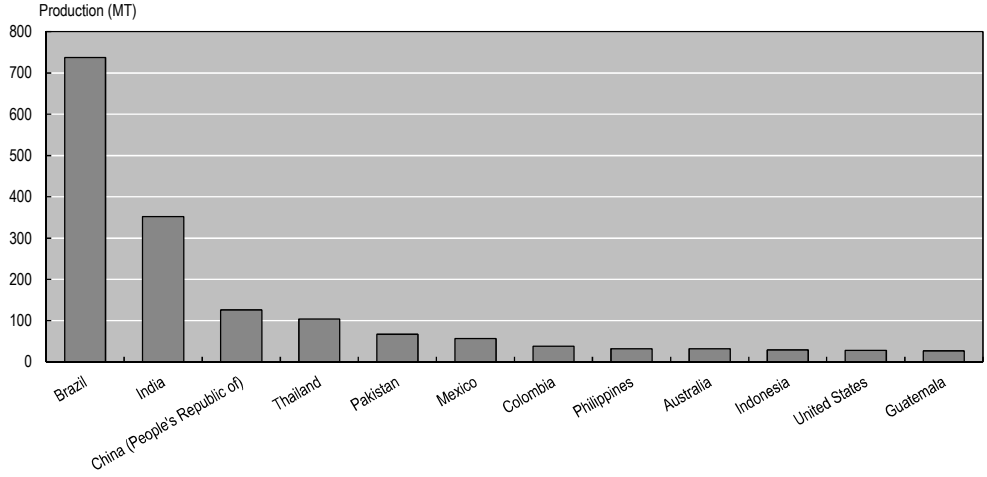
In 2014, world production of sugarcane was estimated to be about 1 900 million t, which was grown on approximately 27.2 million hectares. Brazil was the largest producer at 737 million t (FAOSTAT, 2014). The world production of sugar from sugarcane is approximately six times that from sugar beet, the other major source of sugar.

Figure 2.1 shows the production of the first 12 sugarcane producing countries in 2014. Other countries which were part of the top 20 producers in the same year included (in descending order of output) include Argentina, Viet Nam, South Africa, Cuba, Egypt, Peru, Myanmar and Ecuador (FAOSTAT, 2014).

Sugar production

Sugarcane is an established agricultural field crop with a long history of safe use. The process for extracting sugar from sugarcane is outlined below, and described in more detail in the OECD sugarcane document that deals with the safety of novel food and feeds (OECD, 2011).

Figure 2.1. **Sugarcane production by top 12 producing countries in 2014**



Source: Compiled from FAOSTAT (2014).

Sugar is initially extracted from the raw cane at sugarcane mills distributed throughout the growing regions. The cane is shredded and the juice extracted by crushing. The juice is then clarified by heating in the presence of lime ($Ca(OH)_2$). The lime complexes with phosphorus in the juice to produce a precipitate of calcium phosphate, which is allowed to settle out taking other impurities with it. Flocculants are added to speed up this precipitation process (Mackintosh, 2000). In some production schemes sulphur dioxide and small quantities of soluble phosphate may also be added to decrease juice viscosity and minimise colour development (Andrews and Godshall, 2002).

Clarified sugar juice is concentrated by evaporation to produce "syrup". The syrup then goes through multiple rounds of crystallisation to extract the sucrose. The syrup is boiled and the sucrose crystallises from the remaining molasses fraction as it cools. This mixture is known as massecuite, and the sugar crystals are separated from the molasses by centrifugation. This process is repeated three times. Thus, clarified sugar juice is boiled and centrifuged the first time to produce "A" sugar and "A" molasses. "A" molasses is then boiled again to produce "B" sugar and "B" molasses. The "B" molasses is boiled a third time to produce "C" sugar which is mixed with water and is used to seed the next round of crystallisation (Mackintosh, 2000). The "C" molasses is referred to as "final" or "blackstrap" molasses (Preston, 1988). The "A" and "B" sugars are dried to produce raw sugar. This may be consumed locally or shipped in bulk to sugar refineries worldwide for further refining, resulting in a highly purified product.

Sugarcane payment schemes

The method for calculating payment for sugarcane varies, although in many countries cane payment is based on the quality of the sugarcane (Lejars et al., 2010). In other countries including China, Pakistan and parts of India growers receive a fixed price per tonne (Todd, Forber and Digges, 2004).

In some countries such as Australia, Jamaica, Mauritius and South Africa there is compensation for yield and quality if cane is delivered at the beginning or end of the season to encourage growers to extend their harvesting period to extend the milling season. In other countries such as Brazil, the millers process their own cane in these off-peak periods (Todd, Forber and Digges, 2004).

Sugarcane quality may be measured at the mill. The formula to determine payment to the grower is complex and varies between countries; however, payment often uses two measures of cane quality, Brix and Pol. Brix is the percentage of dissolved solids on a weight per weight basis and is measured by refractometer or density meter. Pol is a measure of the degree of rotation of polarised light through a known quantity of clarified juice, which estimates sucrose content. In Japan, the Pol% cane is measured and a premium or reduced price is paid for the cane depending on whether this is higher or lower than the standard (13.1-14.3%) (Matsuoka, 2006). In Australia, Brix, Pol and fibre content are used to estimate the extractable sugar content or commercial cane sugar (CCS) of a grower's cane (Mackintosh, 2000), which determines the payment. The average CCS in Australia is around 13%, but can be as high as 18% (Jackson, 2005). A similar system in Louisiana (United States) and South Africa uses Brix, Pol, percent fibre and percent sediments to determine theoretically recoverable sugar (TRS) or recoverable value (RV), which forms the basis for grower payments (Dalley and Richard, 2010; Wynne, Murray and Gabriel, 2009). In Brazil before 1997, the government set sugarcane prices prior to harvest, but since deregulation, most of the mills use a payment system based on TRS (Valdes, 2011). The commercially recoverable sugar, which is actually recovered by the mill, varies depending on the mill efficiency, but is usually 95% of TRS (Dalley and Richard, 2010).

Other products from sugar production

Several other products are produced from crushing sugarcane at the sugar mill. In Cuba, it has been estimated that up to 31 products are produced from sugarcane. These include refined sugar, raw sugar, molasses, alcohol, rum, bagasse, syrups, dextran, confectionary, crude wax and glucose. One hundred tonnes of sugarcane is estimated to produce 14.3 t raw sugar, 27.2 t bagasse, 5.2 t filter cake, 2.6 t molasses and 50.7 t waste water (Allen et al., 1997).

Ethanol

In most countries, some of the sucrose is fermented to produce ethanol (Schubert, 2006). In 2006 in Brazil, 47% of the sugarcane crop was used for ethanol production, yielding 17.8 billion litres (summarised in Goldemberg and Guardabassi, 2009) and providing around 40% of fuel used in cars in Brazil (Orellana and Neto, 2006). In the 2010/11 season, ethanol production from sugarcane increased to 54% of the crop, producing 27.6 billion litres of ethanol (Conab, 2011). The residue of the fermentation, called vinasse, is used as fertiliser in fields in Brazil (Cheavegatti-Gianotto et al., 2011). In Thailand there are 12 ethanol production plants with a production capacity of 1.7 million litres/day, but in 2009/10 sugarcane was primarily used for sugar production (USDA FAS, 2009). In India, molasses is used to produce 3.2 billion litres of ethanol/year in 300 distilleries (Gopinathan and Sudhakaran, 2009).

Sugarcane juice is also fermented and distilled to produce alcoholic beverages such as *cachaça* in Brazil or rum (although in some countries this is made from molasses).

Bagasse

Bagasse is the fibrous portion of sugarcane that remains after the juice has been removed. It is estimated that 240-280 kg of bagasse is produced for each tonne of sugarcane processed (Cheavegatti-Gianotto et al., 2011). Bagasse consists of two types of fibre: the long fibres in the rind, and the shorter, softer fibres in the pith of the cane stem.

Bagasse cellulose fibres are longer (1-1.5 mm) than hardwood fibres (0.7-1 mm), but shorter than softwood fibres (2.5-5 mm) and are suitable for papermaking. Bagasse is used to make paper in many countries (Allen et al., 1997; Almazan, 1994). The pith material of the stem, which comprises 25-35 % of the bagasse dry weight, is considered a contaminant and it must be removed for high-quality paper making (Dunlap and Callihan, 1969). Internationally, bagasse has also been used to make particleboard, a construction panel that can be used for cabinets and laminate flooring (Nelson, 1998) and fibre board (Almazan, 1994). More recently, panels have been prepared using bagasse as the basis for both the resin and the fibres in the board (Hoareau et al., 2006).

Bagasse is used as an animal feed but its use is limited by low digestibility, even for ruminants. Steam treatment of the bagasse improves its digestibility so that it can be used in the fattening of cattle (Allen et al., 1997; de la Cruz, 1990; de Medeiros and Machado, 1993; Pate, 1982; Playne, 1984; UN Industrial Development Organisation, 2002). Bagasse has also been used as food for shrimp (Freeman, Duerr and Leber, 1992).

Bagasse is burnt for heat to produce steam as a source of power to run the sugar mills, with excess energy directed to the electricity grid in a number of mills, including those in Australia (Mackintosh, 2000), Brazil (Cheavegatti-Gianotto et al., 2011) and Mauritius (Deepchaud, 2005).

In the future, bagasse may also be used in the production of bio-fuels such as ethanol (Sainz and Dale, 2009).

Bagasse is also an effective bio-sorbent and may be used in waste water management. For example, common pollutants found in synthetic waste water such as chromium, cadmium, copper-nickel and dyes are effectively adsorbed by bagasse (de Matos et al., 2003; Khan and Amin, 2005; Khattri and Singh, 1999; Krishnani, Parimala and Meng, 2004; Sousa et al., 2009).

Molasses

Molasses is the thick syrupy residue left after the sucrose has been removed from the clarified sugar juice (syrup). The “C” molasses (final or blackstrap molasses) is used for alcohol fermentation, as a tock feed supplement and as a fertiliser for cane fields (Mackintosh, 2000; Sansoucy, Aarts and Leng, 1988; Sreenivasan et al., 1987).

Other products

Trash is the plant material left after harvesting of the sugarcane stalks. It is estimated that there are 10 t of trash produced per hectare of sugarcane (Karve et al., 2001). In parts of Australia, trash is generally retained in the field as mulch or it may be baled and used as garden mulch and as a low-grade cattle feed. In India, equipment has been developed to turn the trash into solid briquettes for use as fuel (Karve et al., 2001).

Sugarcane wax comprises both the waxy coating on the outside of the stalk – concentrated mainly at the nodes – and the lipids found throughout the cells (Allen et al., 1997). Sugarcane wax is used in cosmetics and pharmaceutical products, such as in products used to lower cholesterol.

Sugarcane ash (the residue produced when the sugarcane bagasse is burnt as fuel in the boilers) and filter cake or press mud (the solids left after filtering the cane juice) are often used as fertilisers on sugarcane farms (Cheavegatti-Gianotto et al., 2011; Qureshi et al., 2000). It is estimated that 1 t of sugarcane crushed in Queensland,

Australia produces 0.01 t of sugarcane ash and 0.05 t of mill mud (Qureshi et al., 2000). These provide a good supply of many plant nutrients, although nitrogen may need to be added (Calcino, 1994). In Australian banana plantations, sugarcane ash has been shown to enhance the growth of bananas (Broadley et al., 2004), and in Cuba improved sugarcane and maize (corn) growth was seen following ash application (Onelio et al., 2011). In Brazil, sugarcane ash has been used to replace sand in concrete and mortar for construction (Sales and Lima, 2010), and it has been investigated as an adsorbent for dye removal (Kanawade et al., 2010).

There have been some reports that very long chain fatty acids/alcohols (policosanols) from sugarcane wax lower cholesterol in humans (reviewed in Hargrove, Greenspan and Hartle, 2004). However, other studies reported no effects on cholesterol (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2011; Kassis, Kubow and Jones, 2009; reviewed in Marinangeli et al., 2010). Policosanols have also been reported to decrease risk of cardiovascular disease (Janikula, 2002) and may have anti-inflammatory effects (Ledón et al., 2007).

Other beneficial phytochemicals from sugarcane include glycolic acid, which can be used in cosmetics, primarily for skin rejuvenation (reviewed in Allen et al., 1997).

Taxonomy of species

Classification and nomenclature

Sugarcane belongs to the genus *Saccharum* L., traditionally placed in the tribe Andropogoneae of the grass family (Poaceae). This tribe includes tropical and subtropical grasses and the cereal genera *Sorghum* and *Zea* (known as maize or corn). The tribe is further divided into groups, with sugarcane in the Saccharinae Benth. It then may be divided into two subtribes, with sugarcane in the Saccharastra, sometimes called Saccharininae, although this level of group is not an official International Code for Botanical Nomenclature (ICBN) designation (Daniels and Roach, 1987). The taxonomy and phylogeny of sugarcane is complicated as plants from five genera share common characteristics and form a closely related interbreeding group known as the "*Saccharum* complex". The *Saccharum* complex comprises *Saccharum*, *Erianthus* section *Ripidium*, *Miscanthus* section *Diandra*, *Narenga* and *Sclerostachya* (Daniels and Roach, 1987). These genera are characterised by high levels of polyploidy (polyploids have more than two sets of chromosomes) and frequently unbalanced numbers of chromosomes (aneuploidy), making it difficult to determine taxonomy and resulting in many revisions of the taxonomic relationships ((Daniels and Roach, 1987; Sreenivasan et al., 1987). More recent molecular analysis of the genera in the *Saccharum* complex has led to suggestions that the taxonomy should be rearranged as many of the divisions appear to be polyphyletic (Hodkinson et al., 2002).

The genus *Saccharum* traditionally comprises six species: *S. spontaneum*, *S. officinarum*, *S. robustum*, *S. edule*, *S. barberi* and *S. sinense* (D'Hont et al., 1998). However, Irvine (1999) has suggested that the genus should be reduced to just two species, grouping together *S. robustum*, *S. edule*, *S. barberi*, *S. sinense* and *S. officinarum* as the species *S. officinarum* and leaving *S. spontaneum* as a separate species. His proposal was based on the interfertility of the grouped species and the lack of diagnostic characteristics to separate them into individual species. Other authors have suggested that *Erianthus* is a synonym of *Saccharum* and the *Erianthus* spp. should be included in the *Saccharum* genus (Burner and Webster, 1994). This classification is in use in certain jurisdictions (USDA, 2013a).

Saccharum officinarum was named by Linnaeus in 1752 in *Species Plantarum* (Daniels and Roach, 1987). The word *Saccharum* is thought to have been derived from the Sanskrit "*sharkara*" (Ritter, 1841 as cited in Daniels and Roach, 1987). It is also known by the common name of noble cane. Sugarcane is thought to have resulted from complex introgression between *S. spontaneum*, *Erianthus arundinaceus* and *Miscanthus sinensis* (Daniels and Roach, 1987), although some data support it originating from *S. robustum* (as discussed in Amalraj and Balasundaram, 2006). *Saccharum officinarum* has a chromosome number of 2n=80, with a basic chromosome number of ten, making this species octaploid (having eight pairs of each chromosome). However, *S. officinarum* is not a simple polyploid, as it is both an autopolyploid (more than two sets of homologous chromosomes derived from a single species) and also an allopolyploid (possessing two or more unlike sets of chromosomes) (Sreenivasan et al., 1987). *Saccharum officinarum* has chromosomes in common with both of the genera *Miscanthus* and *Erianthus* section *Ripidium* (Besse, McIntyre and Berding, 1997; Daniels and Roach, 1987), although molecular data has suggested that this is due to common ancestry rather than any direct involvement of these genera in more recent introgression (Besse, McIntyre and Berding, 1997; Grivet et al., 2004).

Saccharum spontaneum is a highly polymorphic, disease-resistant, vigorous species with high fibre content. It has 2n=40 to 128 chromosomes and is a complex polyploid with a probable basic chromosome number of 8 or 10 (D'Hont et al., 1996; Panje and Babu, 1960; Sreenivasan et al., 1987). It can be distinguished from the cultivated *Saccharum* by thinner canes and a narrow inflorescence (Purseglove, 1972). Characteristics of the spikelets at the end of the tertiary branches of the inflorescence are also used by taxonomists to help distinguish this species from other *Saccharum* spp.

Saccharum barberi[1] and *S. sinense* have been in cultivation since prehistoric times in northern India and China, respectively. This has led to considerable interbreeding with other genera and species; consequently, these species are thought to be ancient intergeneric hybrids (Daniels and Roach, 1987). *Saccharum barberi* is thought to be the product of *S. officinarum* x *Erianthus* (sect. *Ripidium*) introgression, while *S. sinense* is thought to be derived from *S. officinarum* x *Miscanthus* introgression. Each contains chromosomes homologous to *S. officinarum* and *S. spontaneum* as well as to those from members of the *Erianthus* and *Miscanthus* genera, again indicating the complex origins and inter-relationships within the *Saccharum* genus (Daniels and Roach, 1987).

Saccharum robustum is a wild species. It is thought to have a most recent common ancestor with *S. officinarum* (Brown et al., 2007; D'Hont et al., 1998) and there is some speculation that it may be the product of introgression between ancestors of *S. spontaneum* and *S. officinarum* (as discussed in Daniels and Roach, 1987). It is a diverse riparian species that grows in the wet tropics as a vigorous perennial up to 10 metres tall and is often used for house and fence posts (Bakker, 1999). Two major groups within the species are known, those that have 2n=60 and 2n=80 chromosomes (Daniels and Roach, 1987).

Saccharum edule[2] is morphologically similar to *S. robustum* except that the flower spike or inflorescence is compacted and remains unopened and enclosed inside the leaf sheaths. It is cultivated as a vegetable in the islands of the Pacific and Papua New Guinea, where it is known as "*navisco*" in Vanuatu, "*pitpit*" in Papua New Guinea and "*duruka*" in Fiji (Grivet et al., 2004; Mudaliar, 2007). *Saccharum edule* is thought to be derived from introgression of *S. officinarum* or *S. robustum* with other genera (Daniels and Roach, 1987).

A summary of the members of the *Saccharum* genus is shown in Table 2.1.

Table 2.1. **Members of genus *Saccharum***

Species	Description	Sugar content	Chromosome number
S. spontaneum L.	Wild species	Very low-low	2n=40-128
S. robustum Brandes and Jeswiet ex Grassl	Wild species	Very low	2n=60-200
S. officinarum L.	Noble canes	High	2n=80
S. barberi Jeswiet	Ancient hybrid	Low	2n=111-120
S. sinense Roxb.	Ancient hybrid	Low	2n=80-124
S. edule Hassk.	Cultivated species	Low. Compacted inflorescence, eaten as a vegetable	2n=60-80 with aneuploid forms

Source: Buzacott (1965); Daniels and Roach (1987).

Origin and cultivation

Centre of diversity and domestication

Commercial sugarcane hybrid cultivars have arisen through intensive selective breeding of species within the *Saccharum* genus, primarily involving crosses between *S. officinarum* and *S. spontaneum. Saccharum officinarum* accumulates very high levels of sucrose in the stem but is highly susceptible to diseases (Cox, Hogarth and Smith, 2000; Lakshmann et al., 2005), whereas *S. spontaneum* accumulates little sucrose, has thinner stalks and higher fibre content but is a highly polymorphic species with resistance or tolerance to many pests and diseases (Bull and Glasziou, 1979; Jackson, 2005).

The origins of *S. officinarum* are intimately associated with the activities of humans, as *S. officinarum* is a purely cultivated or garden species which is not found in the wild (Sreenivasan et al., 1987). The centre of origin of *S. officinarum* is thought to be in the Indonesia/New Guinea area (Daniels and Roach, 1987), where it has been grown as a garden crop since 8000 B.C. (Fauconnier, 1993). It has been proposed that *S. officinarum* evolved from the selection of sweet forms of *S. robustum*. The canes may have previously been used for house building, fencing and archery (Daniels and Roach, 1987) and may have been selected with the aid of animals such as pigs or rats that would have a preference for sweeter individual plants (Daniels and Roach, 1987). Its cultivation spread along the human migration routes to South East Asia, India and the Pacific, hybridising with wild canes. It reached the Mediterranean around 500 B.C. (Fauconnier, 1993). From there it spread to Morocco, Egypt, the Syrian Arab Republic, Crete, Greece and Sicily, the main producers until the 15th century, followed by introduction to West Africa and subsequently Central and South America and the West Indies (Fauconnier, 1993). It is thought to have reached Australia in 1788 on the First Fleet, but did not become established until after it was reintroduced in 1817 from Tahiti (Bull and Glasziou, 1979).

The centre of diversity of *S. officinarum* is thought to be in Papua New Guinea (Daniels and Roach, 1987), a view supported more recently by amplified fragment length polymorphism (AFLP) marker analysis (Aitken et al., 2006).

S. spontaneum is believed to have evolved in southern Asia (Daniels and Roach, 1987). It accumulates little sucrose content and has thinner stalks and higher fibre content than *S. officinarum* (Jackson, 2005). *Saccharum spontaneum* is an adaptable species and grows in a wide range of habitats and at various altitudes in the tropics through to temperate regions, from latitude 8°S to 40°N extending across three geographical zones. These are: 1) the east zone which is Burma, China, Japan, Malaysia, the Philippines, Chinese Taipei, Thailand Viet Nam and the South Pacific Islands; 2) the central zone,

which includes Afghanistan, Bangladesh, India, the Islamic Republic of Iran, Nepal, Pakistan, Sri Lanka and the Middle East; and 3) the west zone which includes Egypt, Kenya, Sudan, the United Republic of Tanzania, Uganda and other countries in the Mediterranean (Panje and Babu, 1960; Tai and Miller, 2001).

Geographic distribution

Sugarcane is grown in over 100 countries on all continents worldwide (FAOSTAT, 2013) between latitudes 30°N and 30°S (Bull and Glasziou, 1979).

Commercial hybrid cultivars

Until the end of the 19th century most of the cultivars commonly grown were derived from *S. officinarum*, *S. sinense* and *S. barberi* (D'Hont et al., 1996).

Modern commercial hybrid cultivars of sugarcane are mainly descended from interspecific hybridisation between *S. officinarum* and *S. spontaneum* (Bull and Glasziou, 1979). The basic breeding concept involved the combination of vigorous growth, ratooning ability, and tolerance to abiotic stresses and disease resistance from *S. spontaneum* and high sucrose content from *S. officinarum* (Berding, Hogarth and Cox, 2004). However, other *Saccharum* species have also been used as parents. An analysis of plants used in breeding programmes in the 1980s determined that two *S. sinense*, *S. barberi* and *S. robustum*, 19 *S. officinarum* and "a few" *S. spontaneum* clones had been involved in the breeding of the commercial cultivars available at that time (Roach, 1989). Other authors have suggested that the modern cultivars are founded on only 20 *S. officinarum* and less than 10 *S. spontaneum* derivatives (Patade and Suprasanna, 2008). This interspecific hybridisation has increased the geographic range of economic sugarcane production (Berville et al., 2005).

Interspecific hybridisation between *S. officinarum* as the female parent plant and *S. spontaneum* as the male parent produces progeny that have a triploid chromosome number (2n + n=100 to 130) (Sreenivasan et al., 1987). This arises as the female parent transmits 2n chromosomes whereas the male *S. spontaneum* parent transmits the normal n chromosomes. Asymmetric transmission also occurs the first time that the hybrid is backcrossed to *S. officinarum* (Lu et al., 1994) and is thought to be either through endoreduplication or fusion of two nuclei during meiosis. This phenomenon facilitated the breeding of modern sugarcane cultivars as the "*officinarum*" qualities recovered more quickly in the hybrids, thus requiring fewer rounds of backcrossing to produce high sucrose cultivars (Sreenivasan et al., 1987). The process of backcrossing was termed "nobilisation" by Dutch breeders (Sreenivasan et al., 1987). Estimates of the origin of chromosomes in commercial hybrid cultivars using both genomic *in situ* hybridisation (GISH) and AFLP markers have suggested that approximately 80% are derived from *S. officinarum* and 10% from *S. spontaneum*, with the remainder being recombinant chromosomes from the two species produced by the natural process of synapsis during meiosis (D'Hont et al., 1996; Hoarau et al., 2001). However, a later study on different cultivars, using GISH and other methods, estimated their genetic complement as mainly *S. officinarum*, with approximately 15-20% *S. spontaneum* chromosomes and less than 5% translocated or recombinant chromosomes (Cuadrado et al., 2004).

Hybridisation between *S. officinarum* and *S. spontaneum* culminated with the release of a cultivar called POJ2878 ("Java Wondercane") in 1921 in Java (Indonesia), which became an important cultivar, allowing for a 35% increase in sugar production over the previous best cultivars (Cox, Hogarth and Smith, 2000; Jeswiet, 1929). This cultivar has provided the genetic heritage for many modern cultivars.

There is an international system for naming sugarcane cultivars, co-ordinated by the International Society of Sugar Cane Technologists (ISSCT). This comprises letters and numbers e.g. POJ2878. The first letters relate to the country where the cultivar was first selected and the breeding station, the numbers relate to the year the cultivar was first sown or the selection made, followed by a numerical sequence. For example, POJ refers to "Proefstation Oost Java" Indonesia. There are a number of international collections kept in the Brazil, India, South Africa and the United States to store important cultivars for use in breeding (Fauconnier, 1993).

Cultivation

Cultivation practices vary between countries and even between regions within a country depending on both the natural environment (e.g. climate, soil) and the human environment (e.g. population, history and mechanisation) (Figure 2.2).

Figure 2.2. **Sugarcane growing in Bundaberg, Queensland, Australia**

Source: Courtesy staff at OGTR, taken in 2007.

Commercial propagation

Propagation of sugarcane is different from the majority of other field crops since commercial sugarcane is propagated vegetatively. A variety or cultivar refers to the specific clone or genotype that has been vegetatively propagated through whole stalks or setts (shorter stem segments), also known as billets, seed pieces or seed canes. The term "seed cane" is used to distinguish them from true, sexually produced seed. The planting material is usually grown on-farm as transport is often not practical due to the large volume of material required and the short viability of the harvested cane (three to four weeks). In Australia, primary seed cane is raised in areas approved by the Cane Protection and Productivity Board as being free of disease and this cane is then distributed to the growers who multiply enough cane for their own crop planting (Croft, Magarey and Whittle, 2000). The number of propagules per stalk is about ten (Snyman et al., 2008b), so a large area is needed to grow seed cane. In Japan, 20 000-35 000 two-budded setts are planted per hectare (Matsuoka, 2006). In Brazil this is estimated at 8-12 t per ha of planting cane (Cheavegatti-Gianotto et al., 2011). In Australia, it is estimated that 880 million setts are produced annually for planting (Mordocco, Brumbley and Lakshmanan, 2009). In Brazil, there have been trials of the PLENE™ system which uses 4-centimetre single bud cuttings in conjunction with a mechanical planter. These are coated with chemicals to protect them against pests and diseases. This system uses significantly less planting material than conventional or billet planting systems (Syngenta, 2010).

Commercial sugarcane is also propagated by allowing the regrowth of the stems of the stools that remain in the soil after harvest of the previous crop (ratooning).

In Argentina, in the province of Tucuman, Project Vitroplantas has been using meristem culture and *in vitro* propagation to produce high-quality seed cane since 2000-01 (Sepúlveda Tusek et al., 2008). This has also been trialled in both Australia and South Africa; there have been some trials with sugarcane plants generated through *in vitro* micro-propagation (Meyer et al., 2009; Shannon, Pace and Di Bella, 2008; Snyman et al., 2008b). Micro-propagation of sugarcane provides a reliable and fast method for mass propagation of clonal material. Micro-propagation of meristem tissue has also been used to obtain disease-free planting material (Lakshmanan et al., 2005; Ramgareeb et al., 2010) and this is used in Brazil, the Philippines and parts of India for generating nursery material (Irvine, 2004; Jalaja, Neelamathi and Sreenivasan, 2008). Plants can be regenerated directly from meristem tissue or indirectly (*de novo*) from callus derived from meristem or non-meristematic cells. Thin cell layer culture of immature leaf or inflorescence tissue can also be used for the direct regeneration of plants (Lakshmanan et al., 2005; Snyman et al., 2006), and can be combined with an automated culture system to reduce labour costs (Mordocco, Brumbley and Lakshmanan, 2009). Plants generated through *in vitro* propagation may show phenotypic variation, although in some cases this is transient and may be due to epigenetic effects, possibly caused by *in vitro* stress (reviewed in Snyman et al., 2011).

Scale of cultivation

World average productivity of sugarcane is 61 t cane per ha, which produces an average of 5.82 t sugar per ha (Hussain et al., 2004b). According to the FAO statistical database, the world average productivity in 2014 was of 57.9 t cane per ha (FAOSTAT, 2014), however, with important differences among countries. Table 2.2 shows the range and diversity of yield reported for the top 12 producing countries in 2014. In 1999, Australia had the highest productivity at 88.97 t cane per ha (Baldani et al., 2002). In the period 1990-95, the highest average sucrose yield for the Queensland (Australia) sugarcane industry was 12 t sucrose per ha, with the highest maximum sucrose yield of the Burdekin region in Queensland, at 17.4 t sucrose per ha (Berding, Hogarth and Cox, 2004).

Table 2.2. **Sugarcane yield in top 12 producing countries in 2014**

Country	Cane production (million tonnes)	Area harvested (hectare)	Yield (tonnes of cane per hectare)
Brazil	737.2	10 437.6	70.6
India	352.1	5 012.0	70.3
China (People's Republic of)	125.6	1 738.1	72.3
Thailand	103.7	1 353.0	76.6
Pakistan	67.5	1 173.0	57.5
Mexico	56.7	761.8	74.4
Colombia	38.2	404.5	94.3
Philippines	32.5	432.0	75.1
Australia	30.5	375.0	81.4
Indonesia	28.6	472.7	60.5
United States	28.0	352.2	79.5
Guatemala	27.4	263.8	103.7

Source: Compiled from FAOSTAT (2014).

In Brazil, sugarcane is grown on approximately 10.4 million hectares (Table 2.2). The majority (70%) is grown in the south-east region, with other sugarcane producing areas in the northeast and midwest of Brazil (CONAB as cited in Cheavegatti-Gianotto et al., 2011). These areas are not generally irrigated, although production is now spreading to drier regions (Cheavegatti-Gianotto et al., 2011). In 2010, the cane crop had an average yield of 79.7 t per ha (Valdes, 2011). In São Paulo, the southeast sugarcane crops are high yielding, some producing over 100 t per ha (Yoneyama et al., 1997). Most of this is used for ethanol production due to the location of the distilleries, with the sugarcane from the northeast region used to produce sugar for export (Bolling and Suarez, 2001).

In Argentina, sugarcane is grown in the north. Production averages 18 million tonnes from 320 000 ha of land, with an average yield of 56 t cane per ha (Ferraro, Rivero and Ghersa, 2009). The FAO estimated the national production in 2014 of 24.6 million tonnes from 386 550 ha, with an average yield of 63.6 t cane per ha (FAOSTAT, 2014). However, average cane yields of 94.5 t cane per ha (from 33 500 ha) were recorded in 2005 from Jujuy Province (Gomez, Chapple and Mcdonald, 2007).

In India, which is the second producing country of the world, sugarcane is grown in both tropical and subtropical regions. The productivity of sugarcane in the tropical belt is 26.4% higher than in the subtropical belt (57.8 t per ha) (Singh et al., 2010b). The sub-tropical state of Uttar Pradesh occupies half of the total area in which sugarcane is cultivated (Gujja et al., 2009). The highest productivity is achieved in the tropical state of Tamil Nadu, with 105 t cane per ha, but the average productivity is low in India, with some regions producing only 40 t cane per ha (Gujja et al., 2009).

In China, sugarcane is grown in the south and southwest regions, with 68% of the production in Guangxi Province (Chen and Yuan, 2010). The average yield from 1990-95 was 58 t cane per ha (Greenfield, 1998). The crop was grown on 1.3 million ha in 2014 with an average yield having increased to 76.6 t cane per ha (Table 2.2).

In Thailand, in 1996 sugarcane was grown on approximately 1 million ha of either irrigated or rain-fed land, with an average yield of 58.7 t cane per ha (Greenfield, 1998). The crop was grown on 1.3 million ha in 2014 with an average yield having increased to 76.6 t cane per ha (Table 2.2).

In Pakistan, sugarcane is grown on about 1.2 million ha (Table 2.2), with 65% of this in the Punjab Province. According to Greenfield (1998), the average sugarcane yield was 46 t cane per ha, although with variation between regions. The FAO reports an average yield for the country having increased to 67.5 t cane per ha in 2014 (Table 2.2).

In Africa, South Africa is the largest producer, with sugarcane grown on 413 000 ha in 2008-09, predominately in KwaZulu-Natal (South African Sugar Association, 2011) while the FAO reports a total country acreage of 312 590 ha in 2014 (FAOSTAT, 2014) The following countries having important sugarcane acreages in the region are Egypt with 140 900 ha and Cameroon with 131 770 ha (FAOSTAT, 2014)

Sugarcane is grown in the United States in the southern states of Louisiana, Florida, Texas and in Hawaii (Greenfield, 1998). In the period 2002-07, the number of farms growing sugarcane in the United States decreased from 953 to 692, but the average area harvested per farm increased from 415 ha (1 027 acres) to 495 ha (1 224 acres) per farm (USDA ERS, 2013). It is also grown in 15 of the 23 states in Mexico, which ranked as the sixth global producer in 2014 (FAOSTAT, 2014). In Mexico, the average cane yields are 74.4 t per ha (Table 2.2), although this is variable depending on rainfall and region. The

industry has a large number of small growers, with each mill dealing with cane from 2 500 growers with an average of 6.4 ha each (Buzzanell, 1998).

Sugarcane is also grown on the two main islands of Fiji. In Indonesia, 75% of the 472 700 ha is grown on Java. In 1995, Malaysia had a small industry (approximately 20 000 ha) (Greenfield, 1998), with a larger industry in the Philippines (approximately 432 000 ha) and in Viet Nam (approximately 305 000 ha) (FAOSTAT, 2014). In the Philippines, sugarcane is grown in 17 provinces on 6 islands across the country, with 55% grown on Negros island (Zabaleta, 1998). Japan has a small sugarcane industry, with about 22 900 ha spread across the south-western islands, having produced an average of 89.72 t cane per ha in 2014 (FAOSTAT, 2014).

The scale of sugarcane farms varies both between and within countries. For example, on Réunion Island, the average farm size is 5 ha, which produces an average of 70 t per ha sugarcane, giving the island a total production of approximately 2 million t of cane (Lejars and Siegmund, 2004). Similarly, in South Africa, 43 500 of the 45 300 registered growers have less than 10 ha of land for growing sugarcane and produce only 11% of the crop (Snyman et al., 2008a). In the Philippines, 80% of the 41 000 farmers produce 29% of the crop on less than 10 ha of land each (Greenfield, 1998). In Viet Nam, the industry consists mainly of smallholders with between 0.3-1 ha of land (Greenfield, 1998) and in Japan the average farm size is 0.8 ha (Matsuoka, 2006). In China, there are approximately 5 million sugarcane farms, with an average farm size of 0.27 ha (Chen and Yuan, 2010). In India, average farm sizes are less than 1 ha, with only 25% of the farms greater than 4 ha in size (Gopinathan and Sudhakaran, 2009). Conversely, in Australia the size of the farms varies from 40-250 ha (Canegrowers, 2009).

Cultivation practices

Sugarcane will grow on a wide variety of soil types, although heavy soils are preferred (Purseglove, 1972). In the Philippines, it is grown on both sandy and clay loams, acidic volcanic soils and calcareous sedimentary soils (Zabaleta, 1998). In Australia, it is generally grown in fine-textured sandy loam, clay loam and clay soils (Blair and Stirling, 2007). As well as adequate soil fertility, it requires high temperatures and high rainfall (1 525 mm per year) or irrigation (Purseglove, 1972).

Setts are generally planted within a few days of harvest of the cane, in order to achieve a high frequency of germination (sprouting). Sugarcane is planted in a range of row spacing from 60-150 cm. Buds on planted setts, or on the plant bases remaining after harvest, germinate within two weeks. Sugarcane cultivars differ in their degree of temperature sensitivity, but in general germination is slow at soil temperatures below 18ºC and increases rapidly up to about 35ºC (Bull, 2000; Millard, 1974; Oliveira et al., 2001). Alternatively, in south India and Indonesia, single buds are planted out in a nursery and then the resultant young shoots are transplanted to the field. This is often used where the cane is grown in rotation with rice. The lateral buds on the setts are encouraged to germinate then planted out into the fields, ensuring early establishment and allowing extra time for the rice crop to grow (Fauconnier, 1993). Wider row spacing has also been recommended in India to reduce the amount of planting material required, and increase air and initial sunlight penetration into the crop (Gujja et al., 2009).

Cane can be planted mechanically, but manual planting is common in most parts of the world. In 2005 in Florida (United States), 95% of the land was planted manually (Glaz and Gilbert, 2005) and in Mauritius partially mechanised planting is used (Ismael et al., 2008).

Because sugarcane originated in the wet tropics, yields are much higher when the crop is supplied with adequate water, so sugarcane is grown under irrigation where water is available and rainfall is inadequate. It has been estimated that between 89-118 kg of water is required to produce 1 kg of sugarcane in Florida (Shih and Gascho, 1980).

The cultivation of sugarcane relies on the extensive use of fertilizers and pesticides. Nitrogen especially is widely used. Nitrogen is lost to surface runoff, groundwater, soil storage and the atmosphere (Bohl et al., 2000; Freney et al., 1994; Macdonald et al., 2009; Weier et al., 1996). In Australia, there has been a decline in nitrogen usage, from an average of 206 kg N per ha for the 1997 crop to 164 kg N per ha for the 2008 crop (Wood et al., 2010). The introduction of the "Six Easy Steps" approach is intended to reduce this further (Schroeder et al., 2009). A report from Japan suggests that nitrogen is applied at 200-300 kg per ha, phosphorus at 80-120 kg per ha and potassium at 50-120 kg per ha (Matsuoka, 2006). In Brazil, sugarcane is grown with low nitrogen inputs (50 kg per ha) (Boddey et al., 1991), leading to the suggestion that some cultivars of sugarcane can obtain nitrogen via biological nitrogen fixation.

It has been estimated that a crop of 74 tonnes of cane per ha removes 107 kg nitrogen, 60 kg phosphorus oxide and 300 kg potassium oxide per ha (Purseglove, 1972). The sugarcane plant requires nitrogen for optimum development for yield and sugar content of the canes. Symptoms of nitrogen deficiency are thin, stunted stalks; yellowing leaves with necrosis at the edge and tips; and reduced root mass (Calcino, Kingston and Haysom, 2000). However, excess nitrogen can prolong the crop maturation, resulting in a plant with an excessive leafy canopy, which in turn can make the plant more susceptible to leaf diseases and attack by pests (Bakker, 1999). It can also cause excess growth with little storage of sucrose (Irvine, 2004).

Phosphorus is required for optimum growth. Deficiencies may manifest as plants with short, thin stalks and stools with a low number of primary stalks, a poorly developed root system and sometimes leaves that are green-blue in colour. Conversely, an excess of phosphorus can lead to a deficiency of other trace elements such as zinc and iron, thus reducing sugar yields (Bakker, 1999).

Potassium is required for many physiological processes. It helps to promote the formation and translocation of sugars, and thus may improve the extraction and purity of the cane juice. Supplementing sugarcane plants that are exposed to excessive nitrogen with potassium can alleviate the symptoms of over-supply of nitrogen. Potassium deficiency results in depressed growth, thin stalks and yellowing of the older leaves with chlorotic spots and ultimately death of the leaf (Bakker, 1999). Potassium may also play a role in the ability of sugarcane to withstand dry conditions (Wood and Schroeder, 2004). An excess of potassium increases the ash content of sugarcane juice and reduces the recovery of sugar, and, as with phosphorus, it may also lead to a deficiency of other trace elements (Calcino, 1994).

Calcium is an important element for plant growth and also a regulator of soil acidity. A deficiency in calcium results in leaf chlorosis and reduced stem diameter. Increasing soil acidity, which can be ameliorated by lime application, can result in an increased fixation of phosphorus, aluminium, iron, manganese and nickel, which may lead to toxicity (Bakker, 1999).

Magnesium is important for photosynthesis, being required for chlorophyll function, and is responsible for the green colour in the leaves (it absorbs the blue and red light spectrum). Deficiencies result in leaf chlorosis and stalks of reduced diameter with internal browning (Bakker, 1999).

Other micro-element requirements include sulphur, iron, aluminium, zinc, copper, boron, silicon, molybdenum and manganese. Both deficiencies and toxicity to these elements can occur, resulting in symptoms such as reduced growth, reduced root development and a reduction in photosynthesis (Bakker, 1999).

Agricultural chemicals are widely used to protect the crop from a range of pests and diseases and to control weeds. In Australia, it is estimated that herbicides comprise 90% of the pesticides used on sugarcane farms (Christiansen, 2000). These are used both within the crop and in other areas on the farm to reduce nesting areas and food sources for rats (Christiansen, 2000). In addition, rodenticides and fungicides are used to control rodent pests and fungal diseases, respectively. Insecticides are also used to control pests. These include controlled release chlorpyrifos and imidacloprid to control canegrubs in Australia (Allsopp, 2010; Robertson et al., 1995) or carbofuran to control borers in Florida (Hall, Nuessly and Gilbert, 2007) and Pakistan (Rana et al., 1992). Chemicals may also be used to help ripen the sugarcane and increase sucrose accumulation in the stalk. In 1997, in South Africa, 37% of irrigated crop and 2% of non-irrigated crop were ripened with chemicals (Donaldson, 1999). Herbicides such as Fusilade Super (fluzifop-P-butyl), Gallant Super (haloxyfop-methyl) and Ethrel® ((2-chloro-ethyl) phosphonic acid), a growth regulator, are used in South Africa (Donaldson, 1999), Guyana and Swaziland. In Brazil, MODDUS (trinexapac ethyl), a plant growth regulator, is used. Glyphosate is used in Mauritius and the United States (discussed in McDonald, Morgan and Jackson, 2001), with application rates from 40-180 g per ha although legislation in the United States limits glyphosate use to final ratoon crops in Florida, Louisiana and Texas due to concerns over yield losses (Dusky et al., 1985). Ethrel® and MODDUS are also registered for use in Australia, but Ethrel® is not widely used due to variable yield responses between cultivars and the shorter harvest season (McDonald, Morgan and Kingston, 2000). Studies have shown inconsistent effects of ripeners due to the sugarcane variety, water deficit stress and the combination of chemicals used (Donaldson 1999, 1994; Donaldson and Inman-Bamber, 1982; McDonald, Morgan and Jackson, 2001; McDonald, Morgan and Kingston, 2000).

Planting dates for sugarcane depend on whether or not it is to be irrigated; planting of rain-fed sugarcane depends on the timing of the rain. In India, sugarcane is planted both at the start of the wet season (the eksali crop) and harvested 12 months later, and at the end of the wet season and harvested after 16-18 months (the adsali or monsoon crop) (Fauconnier, 1993). In most countries the plant crop (first crop from a planted sett) is harvested after 14-18 months, and ratoon crops after 12 months. In subtropical regions such as Pakistan and Louisiana, harvesting occurs after ten months, before the first frosts. In other countries such as Peru and South Africa, the sugarcane crop may be harvested at up to 24 months (Hussain et al., 2004b). In the Philippines, the harvest season begins in October-December and ends in May (Zabaleta, 1998). In Australia, sugarcane is harvested after either one or two years, depending on the region (McGuire et al., 2003). In order to keep the sugarcane mills supplied with sugarcane, harvesting is spread over as long a period as possible. In some countries such as Colombia, Kenya, Peru, Uganda and the United States (Hawaii), harvesting occurs almost continuously (Fauconnier, 1993).

Flowering is not desirable in commercial cane as it uses both energy and sucrose and may lead to pithy islands in the stems (Purseglove, 1972). The loss of apical dominance and consequent formation of side shoots leads to a reduction in the sucrose content in the stalk. However, if harvesting occurs within two to three months of flowering, this effect is negligible (Bakker, 1999). In Nigeria, flowering is stated as one of the most important factors responsible for low sugar production (El Manhaly et al., 1984). In Hawaii,

sugarcane is harvested after two years, but flowering may occur twice in this crop cycle, which may lead to losses in sucrose yield (Moore and Osgood, 1989). Consequently, diquat, a herbicide, was used in Hawaii to prevent flowering in commercial sugarcane crops for 15 years, although it has now been superseded by Ethrel® (2-chloroethylphosphonic acid) (Moore and Osgood, 1989). Ethrel® is also used in South Africa and Nigeria as in the latter instance the period between flowering and harvest often exceeds four months (Donaldson and Singels, 2004; Fadayomi, Abayomi and Olaoye, 1995). Trials in Sudan showed increased yields due to the prevention of flowering with ethephon of 30 t cane per ha and 4.1 t sugar per ha (Hardy et al., 1986). Low flowering is selected for in variety development programmes in Brazil (Cheavegatti-Gianotto et al., 2011) due to its effect on sucrose yield.

However, there are some conflicting data on the impact that flowering has on reducing sucrose content in sugarcane stems. As discussed in Moore (1987), some of the conflicting data are due to inappropriate comparisons. Different sugarcane cultivars are affected differently by flowering. Individual plants may flower due to altered physiology, which led to the flowering which complicates any assessment of the impact of flowering. For example, a series of 35 field trials using Ethrel® showed reduced flowering and an overall increase in cane weight and sugar yield. However, there was little correlation between reduced flowering and increased yield due to variability between fields (Moore and Osgood, 1989). More recent data from experimental plots in Australia have shown that cane yield, commercial cane sugar (CCS) and sugar yield all decreased following flowering (Berding and Hurney, 2005). Sugarcane is routinely harvested mechanically by cutting stems close to the ground, or by hand cutting in countries such as Malaysia, the Philippines and South Africa. In South Africa, in 2003, more than 90% of the annual harvest of 20 million t was harvested manually, partly due to the steep slopes used for planting (Meyer and Fenwick, 2003). In Brazil, cane harvesting is either semi-mechanised, where it is hand cut but mechanically loaded, or fully mechanised (Cheavegatti-Gianotto et al., 2011).

Sugarcane is harvested either green or burnt. Burnt cane harvesting was introduced in Australia during the 1940s in response to labour shortages (Christiansen, 2000) and to reduce the incidence of rat-borne diseases amongst cane cutters (Wood, 1991). This remained the main harvesting method in Australia until the 1980s when it was replaced by green cane harvesting and trash blanketing, where trash is left on the ground after harvest (Ridge and Norris, 2000). In Colombia, cane burning stopped in 2000 following pressure from environmental groups (Ellis and Merry, 2004). The amount of burning in the state of São Paulo (Brazil), also decreased by 20% from 2008 to 2009 (Silva et al., 2010) and the introduction of legislation in this area is aimed at discontinuing sugarcane burning by 2021 (Cheavegatti-Gianotto et al., 2011). In Mauritius, cool burning is used in the humid and subhumid areas whereby the cane burning is conducted in the mornings, which reduces emissions and the leaf moisture means that some unburnt green leaves remain (Ismael et al., 2008). In Argentina, over 70% of the sugarcane in the Tucumán Province is harvested green; however, some of the trash is then burnt after harvest (Digonzelli et al., 2011a).

In Australia, green cane harvesting and trash blanketing is known to dramatically reduce soil erosion (Prove, Doogan and Truong, 1995) and subsequent herbicide runoff (Kealley, 2009). However, in some situations trash blanketing can reduce yields. In Zimbabwe, trash blanketing reduced yields under conditions of full irrigation, but increased yields where lower levels of irrigation were used (Gosnell and Lonsdale, 1977). In South Africa, trash blanketing has been shown to result in fewer shoots from ratoons,

but they are thicker and longer than those from burnt plots and so the yield is increased in trashed compared to burnt plots (Thompson, 1966). In Argentina, trash blanketing also led to increased sugarcane production per hectare, but this was attributed to more shoots from ratoons with no changes in stalk weight (Digonzelli et al., 2011b). However, weed abundance was seen to be lower in fields that were burnt before harvest (Ferraro, Ghersa and Rivero, 2012). In Queensland and northern parts of New South Wales in Australia, trash blanketing may increase susceptibility to frosts and slow down the growth of ratoon crops due to decreased soil warming (Kingston, 2000). Studies have also shown autotoxic and allelopathic effects of sugarcane residues which delayed sugarcane leaf development, possibly due to the presence of benzoic acid (Viator et al., 2006). In Brazil, the reduction in burning of sugarcane has led to the increase in populations of spittlebug *Mahanarva fimbriolata* to become an important sugarcane pest (Korndörfer, Grisoto and Vendramim, 2011).

Sugarcane grows perennially and the root system, or stool that remains in the ground, will resprout. Ratoon crops grow faster than the original plant crop. Although several ratoon crops are possible, cumulative stool damage from harvesting and weed control operations and the impact of pests and diseases eventually leads to declining yield. The number of times a crop is ratooned varies worldwide and depends on the cost of replanting verses the declining sugar yield from the ratoon. Farmers may also plough-out ratoons early to plant newer, more productive cultivars (Cadet and Spaull, 2003). In Swaziland, on free-draining clay loam soil under irrigation, over 20 ratoons have been harvested, whereas in smallholder fields in Kenya only 2 ratoons are harvested (Ellis and Merry, 2004). Similarly in Thailand, farmers only grow one or two ratoons (Greenfield, 1998) and in Florida only 13% of the crop was in third ratoon or older in 2005 (Glaz and Gilbert, 2005). In Brazil, under rain-fed conditions, three to six harvests typically occur before replanting (Cheavegatti-Gianotto et al., 2011). In Australia, there is variation between regions (Chapman, 1988), but a maximum of four ratoon crops are typically grown before ploughing out the crop and replanting (Bull, 2000). Ratoons may also be removed by ploughing and treating with herbicide (e.g. glyphosate) (Willcox, Garside and Braunack, 2000).

After ploughing out the previous ratoons, another sugarcane crop may be planted immediately, within four to eight weeks or the ground left fallow. Alternatively, sugarcane may be grown in rotation with another crop. In 2005 in Florida, 63% of the sugarcane was not replanted immediately following the final harvest, but the ground was left fallow or planted with another crop such as sweet corn, rice, snap beans, leafy vegetables or radishes before replanting sugarcane the following season (Glaz and Gilbert, 2005). In Australia, legumes are grown as rotation crops, with sugarcane again planted the following winter (Willcox, Garside and Braunack, 2000).

Rotation with another crop helps to reduce the build-up of disease, may provide nitrogen for the next sugarcane crop and provides ground cover to prevent soil erosion (Garside et al., 2001). Experiments in Australia have indicated that including a legume crop to break the sugarcane monoculture enhances the yield of both the following sugarcane plant crop and the subsequent ratoon crops (Garside and Bell, 2007; Garside et al., 2001). This may be partly due to a reduction in soil nematodes. Experiments in Australia have also shown a reduction in most species of plant parasitic nematodes following soybean rotation, though many of these populations recovered quickly (Stirling et al., 2011). Research in South Africa showed that certain green manure crops reduced the populations of some nematode species but others led to increased nematode populations (Berry and Rhodes, 2006). An experiment in Zimbabwe showed

a reduction in nematode numbers in sugarcane fields following a soybean rotation (Shoko and Zhou, 2009).

Crops may be planted between the rows of sugarcane, and although this has been shown to reduce sugarcane yields, it provides extra income for farmers. These inter-row crops include black beans in Colombia, cucumbers and tomatillos in Mexico, sugar beets in Pakistan, potatoes in Louisiana and radishes in Java (Indonesia) (Irvine, 2004). Trials in India showed high yield when sugarcane was intercropped with rice as it enabled the sugarcane to be planted earlier in the season (Singh et al., 2010b). In contrast, studies in Pakistan showed a higher yield of sugarcane and greater overall income when sugarcane was grown alone compared to intercropping with wheat or lentil (Rasool et al., 2010; Sohu, Abro and Oad, 2010).

In Australia, the sugarcane industry is incorporating controlled traffic and minimum till practices. A single pass of heavy machinery over the planting area has been shown to cause soil compaction (Braunack and Peatey, 1999) and multiple passes reduce crop yields (Garside et al., 2009). The adoption of controlled traffic planting practices, where GPS guidance is used to direct machinery to the same path in the field, enables the planting beds to be kept separate from the vehicular traffic zones and thus avoid soil compaction and stool damage in the growing areas. This results in a reduced requirement to cultivate the beds, which reduces costs and may also reduce weed problems (Garside et al., 2004). Precision agricultural practices such as automatic pilot on machinery and variable rate application of soil ameliorants is also being adopted in the state of São Paolo in Brazil (Silva, de Moraes and Molin, 2011). Minimum tillage is used on sloping land in Mauritius (Ismael et al., 2008) and has been trialled in Thailand with higher yields than no-till or conventional treatments (Grange, Prammanee and Prasertsak, 2010).

Crop improvement

New varieties are generated through breeding programmes, which rely on the maintenance of germplasm stocks for breeding material. Lines with desirable genotypes are used for hybridisations to produce new lines. Sugarcane breeding for improved cultivars is a time-consuming process, taking upwards of ten years from initial crosses to final agronomic assessment of elite cultivars (Cox, Hogarth and Smith, 2000). More recently, breeding has explored a number of traits, including biomass production, stress tolerance, drought tolerance, low temperature stress tolerance and disease tolerance (Ming et al., 2006). There has been little increase in sugar content in modern cultivars (Jackson, 2005). Genetic modification techniques have been developed which may permit more economical and efficient development of novel genetically engineered (GE) sugarcane lines (see below; Lakshmanan et al., 2005). However, at the time of publication of the current volume, the release and commercialisation of GE sugarcane varieties is at the early stages and still very limited in the world.[3]

In India, the cane-growing regions are grouped into tropical and subtropical regions, with distinct agroclimatic regions within these regions. Cane varieties are bred specifically for these locations and none of the varieties are grown across all the regions (Nair et al., 2002). However, there is limited genetic diversity between different sugarcane cultivars. In India, the genetic distance between 28 varieties sampled was only 29% (Nair et al., 2002). Similar studies in South Africa found 10-28% genetic distance between 20 sugarcane hybrids (Harvey, Huckett and Botha, 1994). A study of 40 commercial cultivars grown around the world showed 61% average genetic similarity (Lu et al., 1994).

Breeding

Sugarcane breeding programmes rely on crossing of elite cultivars and usually involve cross-pollination. In the case of self-pollination, the arrows (inflorescence) containing the flowers are covered with bags or are kept separate from other clones (Sleper and Poehlman, 2006).

Lines used in breeding programmes are designated as male or female. The method of designation varies between countries, with some, such as Australia, Barbados and Cuba, relying on aceto-carmine or iodine staining to determine the relative amount of viable pollen produced (Cox, Hogarth and Smith, 2000; McIntyre and Jackson, 2001). Results of acetocarmine staining showed a good linear relationship with pollen germination from 20-100% staining (Midmore, 1980). Cultivars with <10% pollen viability are designated female and cultivars with >20% viable pollen (or 25% in Barbados) are designated male. Cultivars with intermediate levels of viable pollen (10-20%) are classified as bisexual and may be used as either male or female parents (McIntyre and Jackson, 2001). In other countries, staining for viability is used, but the amount of viable pollen allowed for a female is higher at 15-20% viable pollen (Guadeloupe) or less than 30% viable pollen (South Africa) (Zhou, 2013). In Florida and Louisiana, visual examination is used to determine pollen production, with females showing closed yellow anthers with no pollen on the stigmas (McIntyre and Jackson, 2001).

Emasculation using hot water or reduction in pollen viability by growing plants at low temperatures has been exploited to produce male sterile plants to use as female parents in breeding programmes (as discussed in Heinz and Tew, 1987; McIntyre and Jackson, 2001).

Crosses may be set up as polycrosses or biparental crosses. Polycrosses, or "melting pot crosses", involve crosses between several elite cultivars with an unshielded pollen source. Polycrosses are thought to be easier and more cost-effective (Berding, Hogarth and Cox, 2004), but there is lack of genetic control and limited information available on parentage (Tew and Pan, 2010).

Sugarcane breeding programmes are severely limited by the nature of flowering of each sugarcane cultivar, particularly by a decrease in flowering and pollen viability at high latitudes (Moore and Nuss, 1987). Crosses can be made only between cultivars which have overlapping flowering periods. Various techniques have been developed to induce flowering including alteration of photoperiod so that flowers can be available for crossing when required (Bull and Glasziou, 1979). However, methods used to alter flowering time may also impact on fertility (Midmore, 1980).

Commercial breeding programmes produce assisted crosses between *Saccharum* spp. hybrids under highly favourable conditions. In one method, flowering stalks are cut off and maintained in buckets of crossing solution. The crossing solution consists of a dilute mixture of acids which help preserve the stalks and provide some nutrients (Cox, Hogarth and Smith, 2000). Male and female arrows are set up inside canvas lanterns (pollen impervious canvas bags) with the male set above the female to allow pollen to be shed downwards onto the female flowers (Cox, Hogarth and Smith, 2000). Once pollinated, the stalks are kept in the bucket of crossing solution and allowed to mature, a process taking 12-14 days (Buzacott, 1965). Marcotting or air layering of sugarcane stalks is also used to maintain stalks for crossing (Bischoff and Gravois, 2004). In more temperate climates, crossing houses with controlled temperature, light and humidity are used to perform specific crosses.

Figure 2.3 illustrates some of the steps involved in this process.

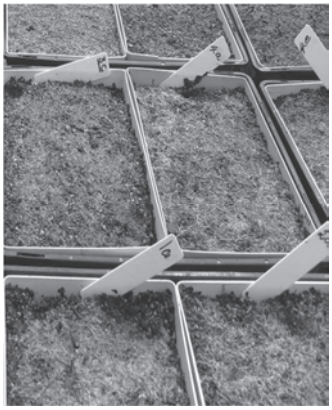
Figure 2.3. **Steps involved in artificial crosses performed in *Saccharum* breeding programmes**

a) Sugarcane cultivars in the glasshouse ready for crossing

b) Cut male and female inflorescence bagged for crosses (2 weeks)

c) Fuzz developing for future seed harvest

d) Sugarcane seeds and small seedlings



e) Sugarcane seedlings in growing trays

f) Sugarcane seedlings in a field trial

Source: Courtesy staff at OGTR, taken in 2007.

To improve the efficiency of breeding and to reliably identify cultivars, modern molecular techniques are being used. Molecular markers can be used to tag genes which are associated with traits of interest, or used to better understand the diversity in the

parents used for breeding (Alwala et al., 2006; reviewed in Hotta et al., 2010; reviewed in Manners and Casu, 2011). Molecular markers have been identified for sugarcane for use in breeding and to identify genetic diversity (Alwala et al., 2008; Heller-Uszynska et al., 2011; Lakshmanan et al., 2005; McNeil et al., 2011; Selvi et al., 2003; Singh et al., 2010a).

Expressed sequence tags (ESTs) have been identified in South Africa from sugarcane meristematic tissue (Carson and Botha, 2000), in Australia and South Africa from sugarcane stem tissue (Carson and Botha, 2002; Casu et al., 2004, 2003) and in India from red-rot infected sugarcane (Gupta et al., 2010). A Brazilian consortium has developed an EST programme (SUCEST) which produced 238 000 ESTs from 26 cDNA libraries, covering different developmental stages and different organs and tissues (Arruda, 2001). ESTs have also been generated in the United States and compared with *Sorghum* and Arabidopsis EST libraries to look for common genes (Ma et al., 2004). These EST projects aim to help expand the knowledge of sugarcane biology and genomics by providing the sequences and possible functions of large numbers of genes that could be related to economically important traits.

In sugarcane, many traits are quantitatively inherited, so quantitative trait loci (QTL) markers are being developed for use in breeding programmes. QTLs have been obtained which are associated with stalk number and suckering (Jordan et al., 2004), sugar content (Aitken, Jackson and McIntyre, 2006; Hoarau et al., 2002; Ming et al., 2002) and yield-related stalk traits (such as stalk weight, stalk number and stalk diameter) (Aitken et al., 2008).

An international sugarcane genome sequencing collaboration is also underway to generate sequence data for *S. officinarum*, *S. spontaneum* and a commercial hybrid (Bonnett and Henry, 2011; Souza et al., 2011; De Setta et al., 2014[4]).

Mutation breeding has been used in sugarcane to add to the natural genetic variation (Patade and Suprasanna, 2008; reviewed in Snyman et al., 2011). This includes experiments using tissue culture to induce somaclonal variation (genetic or epigenetic variation). Somaclonal variants for resistance to eyespot disease (*Helminthosporium sacchari*) have been generated through the screening of plants after tissue culture (Larkin and Scowcroft, 1983). In some instances, selection for the desired trait has been used for example using eyespot toxin or for smut resistance (Rodriguez et al., 2001 as cited in Patade and Suprasanna, 2008). Mutagenesis has also been induced in tissue culture using radiation to produce plants with red-rot resistance, tolerance to water logging, delayed flowering and altered timing of maturity (reviewed in Patade and Suprasanna, 2008) and resistance to downy mildew and improved cane and sugar yield (reviewed in Larkin and Scowcroft, 1981).

Genetic modifications

Sugarcane has a highly complex genome and is vegetatively propagated. This has limited opportunities for crop improvement through conventional breeding of sugarcane (Lakshmanan et al., 2005) and genetic engineering is seen as an important alternative approach for the introduction of new traits. For an overview of methods and target traits for genetic modification of sugarcane see Brumbley et al. (2008).

Sugarcane can be genetically engineered by microprojectile bombardment (Bower and Birch, 1992), electroporation (Arencibia et al., 1995) or *Agrobacterium*-mediated transformation (Arencibia et al., 1998). Positive selection, using the phosphomannose

isomerase/mannose-selection system, has been used to produce GE sugarcane plants that do not contain an antibiotic resistance selectable marker gene (Jain et al., 2007).

Data show that introduced genes are stable in sugarcane and continue to be expressed after asexual and sexual propagation (Hansom et al., 1999; Harrison et al., 2001). However, there is some evidence from field-grown GE sugarcane that yield and CCS is reduced, which may be due to the effects of biolistic introduction of DNA into callus. Controls, which had been through the tissue-culture process but were not subjected to biolistic bombardment (i.e. not genetically engineered), performed better than the GE plants, but still showed reduced agronomic performance. This somaclonal variation is commonly observed after plant tissue culture, is not species specific and is irrespective of the morphogenic route or explant used (Larkin and Scowcroft, 1981).

Although some studies show that a reduced performance of the GE plants compared to controls persisted after ratooning (Arencibia et al., 1999; Gilbert et al., 2009; Vickers et al., 2005b), other field experiments have shown that the phenotypic variations in tissue-cultured sugarcane were temporary and some variants reverted to the original parental phenotype in the first ratoon crop (Burner and Grisham, 1995; Irvine et al., 1991; Lourens and Martin, 1987).

Somaclonal variation from *in vitro*-derived sugarcane has been consistently observed, particularly when plants are produced via a callus stage, which involves long exposure to high levels of certain plant growth regulators (Burner and Grisham, 1995; Irvine, 1984; Irvine et al., 1991; Larkin and Scowcroft, 1981; Lourens and Martin, 1987; Zucchi et al., 2002). The *in vitro* component of the sugarcane transformation process has the potential to generate somaclonal variation to the regenerated plants, and selection by antibiotics or herbicides can add to this increased polymorphism (Carmona et al., 2005). However, as discussed below, the effect may be epigenetic and, in addition, plants exhibiting tissue-culture-derived somaclonal variation are systematically culled during micropropagation-based seedling production systems.

Transposable elements, natural DNA sequences which cause mutations by moving within the genome, have recently been identified in sugarcane (de Araujo et al., 2005). These are expressed mainly in callus and may be the cause of the observed high somaclonal variation in this tissue (de Araujo et al., 2005). Epigenetic effects may also account for observed unusual growth patterns; however, these are often temporary and are usually resolved within a few generations of vegetative reproduction (Birch, 1997; Taylor et al., 1995).

To date, experimental work to genetically modify sugarcane has involved a range of traits including herbicide resistance (Enríquez-Obregón et al., 1998; Leibbrandt and Snyman, 2003), resistance to pests and pathogens (Arencibia et al., 1999, 1997; Arvinth et al., 2010; Braga et al., 2003; Hansom et al., 1999; Ingelbrecht, Irvine and Mirkov, 1999; Joyce et al., 1998; Kalunke et al., 2009; reviewed in Srikanth, Subramonian and Premachandran, 2011; Weng et al., 2006), reduction of browning of sugarcane juice (Vickers et al., 2005a; 2005b) and resistance to drought stress (Molinari et al., 2007).

Sugarcane has also been genetically engineered for the production of novel industrial compounds. Sugarcane is a C4 grass so it has a high growth rate and efficient carbon fixation. In addition to the C4 qualities, it has a substantial carbon flux through metabolic pathways, and the waste bagasse could be used to generate electricity needed for processing of the biofactory products (Twine, 2005). For example, GE sugarcane has been modified to produce altered sugars such as trehalose (Hamerli and Birch, 2011;

Zhang et al., 2006), isomaltose (Wu and Birch, 2007) and sorbitol (Chong et al., 2007) or industrial compounds such as poly-3-hydroxybutyrate (PHB) (Brumbley et al., 2002; Purnell et al., 2007) and p-hydroxybenzoic acid (pHBA) (McQualter et al., 2005). The first field trial in the United States to produce a human pharmaceutical product was conducted with sugarcane genetically engineered to produce human granulocyte macrophage colony stimulating factor (GM-CSF) (Wang et al., 2005).

In Australia, field trials of GE plants with altered sugar production, herbicide tolerance, altered plant architecture, enhanced drought tolerance and nitrogen use efficiency, altered sucrose accumulation and improved cellulosic ethanol production from sugarcane biomass are underway.[5] In Brazil, there have been a number of field trials for traits such as herbicide tolerance, viral resistance, insect resistance, drought tolerance, sucrose yield and inhibition of flowering (Matsuoka, Ferro and Arruda, 2009). In Cuba, field trials of GE sugarcane plants with resistance to insects, fungi and herbicide tolerance have been approved.[6] In the United States, permits have been issued for field trials of GE sugarcane plants with altered sugar storage, resistance to insects, viruses, herbicide tolerance and accumulation of pharmaceutical products.[7] Field trials with GE sugarcane have been conducted in South Africa[8] and the main traits evaluated to date include herbicide tolerance, viral resistance and sucrose metabolism perturbations (Watt et al., 2010). Field trials have been performed in China with GE sugarcane with insect resistance (Weng et al., 2011). In Argentina, field trials have been performed with herbicide-tolerant and virus-resistant varieties (Raney and Matuschke, 2011). At the time of publishing the current volume, the commercialisation of GE sugarcane was still at very early stage globally; one case of GE sugarcane, developed by the Indonesian public research for drought stress tolerance, was approved in Indonesia for food use and cultivation in 2013.[9]

Morphology

Plant morphology

The morphology and anatomy of sugarcane has been extensively reviewed and so will not be explored in great detail here. See Moore (1987), Bakker (1999) and Cheavegatti-Gianotto et al. (2011) for a comprehensive treatment of the morphology and anatomy of sugarcane and Matsuoka and Garcia (2011) for a review of the literature on sugarcane roots.

Sugarcane is a large tropical grass that produces multiple stems or culms, each of which consists of a series of nodes separated by internodes. Following germination (sprouting of sett), the terminal vegetative bud of each shoot lays down a series of nodes. Each node consists of a growth ring or intercalary meristem, the root band (containing root primordia) and a bud above the leaf scar where the leaf sheath attaches, which delimits the node from the internode below. The internodes consist of sucrose-storing parenchyma cells and vascular tissue (Moore, 1987).

The stem of sugarcane is similar to maize (corn) and sorghum in that it is filled with parenchyma cells and is not hollow like many grasses (Griffee, 2000). The stem is the major storage area for photosynthate (sucrose) within the sugarcane plant, rather than fruit or seed structures. Transverse sections through an internode reveal vascular bundles surrounded by parenchyma cells with a thick outer epidermis covered in an external layer of wax. Leaves and internodes develop in a basipetal direction in that the leaf blade expands at the base then the internode elongates. As the stem develops, the leaves

emerge, one leaf per node, attached at the base of the node, forming two alternate ranks on either side of the stem. At the top of the stem is an apical meristem set on top of a number of very short internodes. Mature stems consist of a number of immature leaves still enclosed in the leaf spindle, a dozen or so green leaves and a number of senescent leaves, increasing in number with increasing age of the plant. Leaves may be retained on the stem or they may be shed in some varieties, known as free-trashing. New leaves emerge and expand over a period of between one and three weeks. Internode length can reach over 30 cm, depending on growth conditions, and stems normally reach 2-3 metres in the normal growing season (Bull, 2000; Bull and Glasziou, 1979).

The leaf blade is pubescent (hairy) on the abaxial (under) side of the leaf and glabrous (without hairs) on the adaxial (top) side and terminates in a pointed tip. The leaf blade is 2-10 cm across and 60-150 cm long (Fauconnier, 1993). The base of the leaf blade is attached to the leaf sheath that encloses the internode, joining the stem at the node to which the leaf subtends.

Sugarcane uses a C4 mechanism of photosynthesis similar to other tropical grasses, where the carbon dioxide for photosynthesis is initially fixed by phosphoenolpyruvate (PEP) carboxylase to form a four-carbon compound (Hatch and Slack, 1966). The anatomy of the leaves reflects this underlying physiology; the vascular bundles are surrounded by a ring of bundle sheath cells and a ring of mesophyll cells, an arrangement known as Kranz anatomy.

Like most grasses, the sugarcane root system is fibrous and shallow. It has been estimated that the top 25 cm of soil contains 50% of the plant roots, with the next 35 cm containing a further 40% of the roots (Fauconnier, 1993). However, the effective root zone (i.e. the area of roots which are actively extracting water) varies depending on the soil type, from just the topsoil in sodic duplex soils, to 0.9-1.2 m in irrigated clay loam, to 1.8 m in rain-fed conditions (Ham, McGuire and Kingston, 2000). The root system is dynamic and the area of active root growth varies depending on the irrigation pattern (Inman-Bamber et al., 2008). The plant also develops buttress roots that serve to anchor the plant, and some deeply penetrating roots that grow downwards for up to four metres allowing for water absorption under water stress (Bull and Glasziou, 1979). Roots partially die-back after ratooning, although there is evidence that some roots can persist for at least four months after harvest and some of the new roots emerge from the old pre-harvest roots (Smith, Inman-Bamber and Thorburn, 2005).

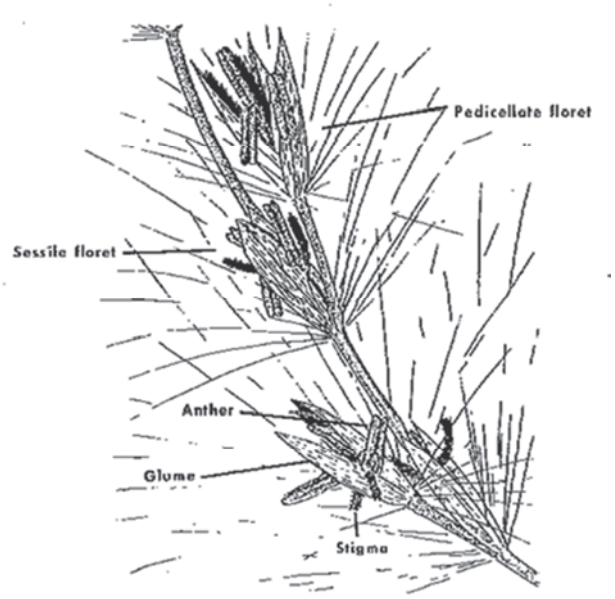
Reproductive morphology

The sugarcane inflorescence is an open branched panicle (a compound raceme), also known as an arrow, whose shape, degree of branching and size are highly cultivar specific (Figure 2.4). The arrow can bear thousands of flowers (Sleper and Poehlman, 2006), and is estimated to average 24 600 florets (Rao, 1980). The arrow consists of a main axis and first-, second- and third-order branches. Attached to the branches are spikelets arranged in pairs, one of which is sessile and one pedicellate, that bear individual flowers (Figure 2.5). At the base of each spikelet is a row of silky white hairs. Sugarcane flowers consist of three stamens (male) and a single carpel with a feathery stigma (female) typical of wind-pollinated flowers. Frequently, the male stamens may be abortive, resulting in reduced or absent pollen production (James, 2004; Moore, 1987; Sleper and Poehlman, 2006). Another colour varies from bright yellow to purple (Moore, 1987).

Figure 2.4. **Inflorescence of *Saccharum* spp. hybrid**

Source: Courtesy G. Bonnett, CSIRO, Australia.

Figure 2.5. **Diagram of a portion of a mature raceme of a sugarcane inflorescence showing the arrangement of sessile and pedicellate spikelets and callus hairs**



Source: Reprinted with permission from Moore (1987). Original figure from Engard and Larsen (1948).

Development

Reproductive biology

Sugarcane can reproduce both sexually and asexually. Sexual reproduction is via true seed, often called fluff/fuzz due to the presence of soft hairs. As discussed previously, the ability of sugarcane to reproduce asexually is exploited for the production of planting material.

Asexual reproduction

Asexual reproduction can occur via nodal buds which are found on setts, via rhizomes or via stools (Amalraj and Balasundaram, 2006). The parent species of *Saccharum* spp. hybrids differ in their ability to form rhizomes and tillers, with *S. spontaneum* forming dense mats of rhizomes and many tillers, whereas *S. officinarum* forms fewer tillers and rhizomes (Amalraj and Balasundaram, 2006; Moore, 1987).

Sexual reproduction

The ability of sugarcane to reproduce sexually was not recognised until the mid- to late 1800s due to its lack of importance as an economic product (Buzacott, 1965). Sugarcane flowering is a complex process consisting of a number of steps which are differentially regulated by photoperiod (Moore and Nuss, 1987), with the early steps having more precise regulation required than the later steps (Midmore, 1980). Flowering is dependent on interaction of genotypes and environmental factors such as day length and temperature.

Flowering is reliable and 80-100% of stalks produce flowers in tropical environments such as Malawi and Sudan (12-13° latitude), whereas it is sporadic at higher latitudes in sub-tropical environments such as South Africa (Donaldson and Singels, 2004). Flowering in the northern hemisphere is earliest closest to the equator (around the autumn equinox in mid-September). At higher latitudes it occurs later, with the peak in October in Coimbatore (India) and Barbados, November in Hawaii and December in southern Florida. In the southern hemisphere, flowering takes place from March through to June (Moore and Nuss, 1987), although flowering does occur outside this peak period (Bonnett et al., 2007). The flowering date of a particular cultivar varies by only a few days between years in the same environment (Midmore, 1980). Some cultivars can flower profusely in their natural environment but only sparingly when introduced to other regions (Bull and Glasziou, 1979). When grown together, cultivars that were selected for use at high latitudes usually flower earlier than those which originated at lower latitudes, suggesting that they require longer day lengths for floral initiation (Moore and Nuss, 1987). Experiments have also indicated that early flowering cultivars often flower more profusely than later flowering ones (Moore and Nuss, 1987).

Floral development is induced by photoperiods of approximately 11.5 hours, which often coincides with a natural day length of 12.5 hours. As a result, the period of floral initiation is more defined further from the equator (Bakker, 1999). Annual variations in flowering times in a given location are mostly attributable to differences in night temperature (Bakker, 1999). Cool night temperatures, high day temperatures and lack of moisture interfere with flower initiation. The older and more vigorous stems in a stool are the most likely to initiate flowering (Moore and Nuss, 1987). Flower initiation causes the apical meristem to switch from vegetative to floral development. Consequently, flowering of the crop can adversely affect yields (Bakker, 1999).

Pollen dispersal and pollination

Sugarcane spikelets open from the top of the panicle, with the outermost spikelets opening first. It takes 5-15 days for all the spikelets on the panicle to open. Spikelets open at sunrise, with anther dehiscence occurring about three hours later, although this is delayed by high humidity (Purseglove, 1972).

Sugarcane pollen grains are very small, hairy and wind dispersed. The round-ellipsoidal grains vary in size from 38.25 µm x 42.75 µm to 67.5 µm x 72.0 µm and are yellow in colour (Dutt, 1929).

Little data is available on sugarcane pollen viability under natural conditions. In Australia, studies have shown that pollen viability from commercial sugarcane fields varies between regions and cultivars, showing a range from 1.2-4.4% viability (Bonnett et al., 2007). Sugarcane pollen begins to lose viability rapidly in less than 30 minutes (Venkatraman, 1922). *S. spontaneum* pollen is rapidly desiccated after dehiscence, having a half-life of only 12 minutes, and is no longer viable beyond 35 minutes under unmodified environmental conditions (26.5°C and 67% relative humidity) (Moore, 1976). At higher humidity the pollen longevity was increased (Moore, 1976). Tests with another cane cultivar (Saratha Desi, which is thought to be derived from *S. barberi*) indicated that pollen viability was maintained for two hours in the lab, or one hour when exposed to sunlight (Dutt and Ayyar, 1928). Sugarcane pollen stored at 4°C under 90-100% relative humidity retains some viability for up to 14 days (Moore and Nuss, 1987).

Little data is available on sugarcane pollen dispersal. Information from breeding work in which plants were isolated by 20 m in open forest has shown that viable pollen is dispersed over this distance (Skinner, 1959). From this work, it was suggested that to prevent contamination of controlled crosses, plants should be isolated by 100 m in open forest or 300 m in open ground (Skinner, 1959).

Sugarcane is a cross-pollinating species, although selfing occurs at low levels (McIntyre and Jackson, 2001; Moore and Nuss, 1987; Tew and Pan, 2010). Sugarcane produces protogynous flowers, where the pistil matures before the anthers. Thus, an individual flower may be cross-pollinated prior to pollen shed from its own anthers (James, 2004). In seven experimental polycrosses, the selfing frequencies ranged from 0% to 45%. Progeny resulting from crosses with a high degree of self-pollination had a reduced ability to survive the winter, suggesting reduced vigour (Tew and Pan, 2010). The reduction in vigour following self-pollination has been observed previously (Skinner, 1959).

Sugarcane flowers often have reduced male fertility or are male sterile and some are self-sterile (Skinner, 1959).

Fruit/seed development and dispersal

After fertilisation, it takes approximately three weeks for the fruit to mature and to be shed (Purseglove, 1972). The seed at the top of the panicle, which was fertilised first, is also the first to mature (Breaux and Miller, 1987). These seed are shed as the inflorescence starts to disintegrate, before the seeds at the base reach maturity (James, 1980). The mature fruit contain whorls of silky hairs at the base and are adapted for wind dispersal (Purseglove, 1972) (Figure 2.6). No further information has been found in the literature on seed dispersal.

Mature fuzz consists of the mature dry fruit (caryopsis), glumes, callus hairs, anthers and stigma (Breaux and Miller, 1987). The additional parts of the inflorescence are generally handled, stored and sown with the seed because it is not practical to separate them. Although many commercial cultivars of sugarcane can produce seed, it is only used in breeding programmes, because the proportion of sugarcane seedlings with agronomic qualities near to those of the parental commercial cultivars is extremely low.

The naked seed (without fuzz) has been measured as 1.5±0.03 x 0.64 ±0.005 mm and weighing 0.54±0.05 mg, which is approximately 1 850 seeds per g (Rao, 1980). One of the sugarcane parent species, *S. spontaneum*, has seed which weighed 0.39 mg with fuzz, or 0.25 mg defuzzed (Ellis and Hong, 2007). In a crossing experiment, up to 30% of the seeds produced were smaller than average or shrivelled; however, many of these abnormal seeds still germinated (Rao, 1980).

Figure 2.6. ***Saccharum spontaneum* seed**

Source: Courtesy K. Saltonstall, Smithsonian Tropical Research Institute, Panama.

Data from crosses have suggested that a low percentage of florets set fertile seed. One estimate of seed germination showed a maximum of 17.2% in a "very heavy" germinator (Price, 1961). Another study showed germination rates of between 3.1% and 22.7% (Rao, 1980). In Australia, seed collected from commercial fields had variable germination, ranging from 0-53.3 viable seed per g (approximately 2.9%)[10] depending on the cultivar and growing region (Bonnett et al., 2007). In breeding work in Barbados, seed viability of one commercial cultivar was 266 fertile seeds per g (approximately 14.4%)[3] (Midmore, 1980).

Seed germination

Some wild species of sugarcane such as *S. aegyptiacum* (now classified as a subspecies of *S. spontaneum*) have significant seed dormancy, whereas some modern cultivars have little seed dormancy (Ellis, Hong and Roberts, 1985; Poljakoff-Mayber, 1959).

Sugarcane seed has short viability even under optimal storage conditions. No data are available on field viability. If stored in polythene at room temperature, fuzz remained viable for 90-120 days (Verma et al., 2002). Artificially dried sugarcane seed lost 90% of its viability in 70 days at 28°C if not desiccated (Rao, 1980). Modelling of seed longevity using data on germination at different temperatures and moisture contents has predicted that under hermetic storage at -20°C, seed from the parent species *S. spontaneum* will not last as long as ten other crop species, with only potato (*Solanum tuberosum*) showing shorter viability (Ellis and Hong, 2007).

Generally, in breeding programmes the fuzz is sown. However, the fuzz can encourage the growth of micro-organisms and a large mass of fuzz can prevent seed contact with the soil (Breaux and Miller, 1987).

Germination of sugarcane seed requires heat and humidity and takes 25 days for small seedlings to appear from seed spread on the soil surface (Buzacott, 1965; Itakura, Kudo and Nakasone, 1980) or lightly covered with peat moss (Zhou, 2013). The optimum temperature for sugarcane seed germination under lab conditions was determined to be 35°C or 38°C (Heinz, 1974; Itakura, Kudo and Nakasone, 1980). A more recent study confirmed that maximum germination was at 36°C, with much less germination at 24°C and none at 12°C. At the upper limit, germination was eliminated at 48°C (Bonnett, 2013).

As the seed germinates, the primary root emerges first followed by elongation of the plumule. The leaves of the plumule then emerge rapidly. Tiller branches emerge from a bud which forms in the axil of each leaf. Adventitious roots form near the leaf bases (Moore, 1987).

The young seedlings are delicate and require optimum temperature, moisture, nutrients and protection from fungal diseases (Breaux and Miller, 1987; Buzacott, 1965). Information obtained from a survey of sugarcane breeders suggests that the conditions required to germinate and grow sugarcane seedlings are exacting (Breaux and Miller, 1987). Constant care and attention are needed to give seeds and seedlings the conditions required for survival, especially in the first three to four weeks post-germination. In Brazil, seed germination is seen in the field in north-east regions when flowering and seed shed occurs in the wet season. In other areas, either the night-time temperatures or soil humidity is too low for successful germination (Cheavegatti-Gianotto et al., 2011).

Viviparity, when the seed germinates before it detaches from the parent plant, has been observed under experimental conditions in both the parent species *S. spontaneum* and in hybrid sugarcane (Ragavan, 1960). It is feasible that moist conditions similar to the experimentally induced ones could occur naturally.

Vegetative growth

As discussed previously, sugarcane is propagated from stem cuttings which are referred to as setts, seed, seed cane or seed pieces (Purseglove, 1972). During the initial stages of germination, root primordia around the nodes of the sett produce a flush of roots, known as sett roots (Bakker, 1999). These roots are not connected directly to the primary shoot but are important in maintaining the moisture in the sett. Following formation of the shoot roots, the sett roots blacken and die (Bakker, 1999). The primary shoot is made up of a number of closely spaced internodes and nodes below ground. Each node develops new bud and root primordia that are the basis of stool establishment. These root primordia germinate to produce the shoot roots that support further plant growth. The shoot is then independent of the original sett (Bull, 2000).

While the shoot roots are developing, some of the new buds below ground also germinate to produce secondary shoots or tillers. These, in turn, develop their own root systems and give rise to shoots (Bull, 2000). Shoots usually appear above the soil approximately 12 days after planting, with the first leaf unfurling approximately 8 days later (Bakker, 1999).

Stem elongation is initially rapid and during this phase the fibre content of the stem is relatively high, whereas the sucrose levels are still quite low.

At maturation, the growth rate slows and sucrose content increases (Bull, 2000). Maturation and ripening are reversible processes and are associated with the lower rainfall and cooler temperatures of the winter months. During stem growth, each internode operates as an independent unit. While it has a green leaf attached, the internode completes cell elongation and cell wall thickening, and fills with sucrose. Hence internodes generally complete their cycle by the time the attached leaf dies, and the lower internodes are essentially ripe while the upper part of the stem is still growing. The stored sugar is, however, available for translocation to support further tillering and/or growth when conditions are not favourable for photosynthesis (Bull, 2000).

As the stem matures, more internodes reach the same condition and sucrose content rises. During this period, the most recently expanded internodes near the top of the stem stop elongating and photosynthates are channelled into storage as sucrose. Factors that affect the maturation of the sugarcane stem include age, nitrogen status and moisture. Environmental factors that can influence sucrose accumulation include water stress, nutrient status and temperature (Bull, 2000).

Genetics

As described in the beginning of this chapter, members of the *Saccharum* genus are genetically complex, showing polyploidy with some autopolyploidy and allopolyploidy.

The sugarcane genome has also been shown to contain many expressed sequences as tandem repeats, introducing further complexity (Butterfield et al., 2004).

The haploid genome size of a number of *Saccharum* spp. has been measured using flow cytometry as 2 547-4 183 Mbp (Arumuganathan and Earle, 1991). DNA measurements from *S. officinarum* and *S. spontaneum* also fall within this range (Butterfield, D'Hont and Berding, 2001). The monoploid genome size is thus estimated at 926 Mbp for *S. officinarum* and 760 Mbp for *S. spontaneum*, approximately the same as *Sorghum bicolor* (760 Mbp). Comparisons with other genomes suggest that it is the amount of repetitive DNA that varies between genomes, with the *Saccharum* genes occupying about 20% of the genome (Butterfield, D'Hont and Berding, 2001).

Abiotic interactions

A recent review has provided an overview of studies on abiotic stress in sugarcane (Azevedo et al., 2011).

Nutrient stress

The cultivation of sugarcane relies on the extensive use of fertilizers. As discussed above, to grow sugarcane successfully requires high inputs. This may also limit its ability to grow outside of cultivation.

Temperature stress

Low temperatures

Sugarcane cultivars differ in their degree of temperature sensitivity, but in general sett germination (sprouting) is slow at soil temperatures below 18°C (Smit, 2011) and the setts may succumb to attack by fungal pathogens before they germinate. Sett germination is increasingly rapid up to about 35°C (Bull, 2000).

Experiments have shown that sugarcane plants grow more slowly and have fewer, shorter internodes and fewer leaves at 15°C than when grown at 27°C. The low temperatures also inhibited sucrose export from the leaves to the stalk so the leaves accumulated sugar and starch (Ebrahim et al., 1998).

Flowering is also affected by low temperatures. Cool night temperatures, high day temperatures and lack of moisture interfere with both flower initiation and sucrose accumulation. Temperatures below 18.3°C are non-inductive for flower development (Coleman, 1963). In temperate South Africa, pollen fertility has been shown to be limited at temperatures below 21°C (Zhou, 2013). In Queensland (Australia), artificially increasing the night-time temperature of sugarcane plants to 22-23°C led to increased and earlier flowering (Berding, 1981). Experiments have also shown that heated pollen lanterns, used for crossing, can increase seed setting, due to improved fertilisation and embryo development (Berding and Skinner, 1980).

Experiments have shown that seed germination is markedly reduced at temperatures below 30°C (Itakura, Kudo and Nakasone, 1980).

Sugarcane is susceptible to frost damage (Griffee, 2000). Freezing reduces yields by delaying crop development in spring and by terminating sugar accumulation in autumn (Moore, 1987). In Australia, frost damage is seen in southern areas with about a third of the cane affected by frost, leading to yield losses of 10-30% annually (Weaich, Ludlow and Nielsen, 1993). Frosts may also affect production in southern Florida (Code and Ulloa, 1991). In 2008, almost all of the sugarcane crops in Guangxi Province in China suffered severe cold and freezing injury, leading to a decrease in sucrose content of 0.2-0.5% in plant cane, with a larger decrease in ratoon cane (Tan et al., 2010). Frosts are also a problem in high altitude regions in the Midlands of KwaZuluNatal (South Africa) and Louisiana, leading to early harvesting of the cane due to frost damage (Van Heerden et al., 2009). The degree of damage varies with the severity of the frost. Leaf browning occurs at temperatures from 0°C to -2°C, with temperatures down to -4°C causing damage to terminal and lateral buds and death of some young internodes. If the temperatures reach -11°C, this can cause freezing and subsequent cracking of entire stalks. The cracks or damaged buds can allow entry of anaerobic bacteria such as *Leuconostoc mesenteroides*, which can replicate in the damaged tissues and produce dextran. Dextran intefers with the crystallisation of sucrose at the mill (Irvine, 2004). Frost damage varies between sugarcane cultivars, and this is thought to be due to differences in tolerance rather than differences in morphology, which might protect against frosts (avoidance) (Weaich, Ludlow and Nielsen, 1993). Management practices such as retention of a trash blanket increases the susceptibility to frost by preventing radiation of warm air from the soil (Kingston, 2000).

High temperatures

The literature suggests that sugarcane can survive temperatures as high as 45°C, or higher for short periods of time but growth slows at temperatures above 40°C (Moore, 1987). However, in Iran sugarcane is grown in the Hapft Tappeh region where the average temperature over the summer months is 45.8°C (Sund and Clements, 1974). Sugarcane grown in the Ord River region of Australia, which has mean temperatures in November of 39.4°C (Australian Bureau of Meteorology, n.d.), has been shown to have a lower sucrose content than that grown in cooler regions. Experiments in which sugarcane was exposed to temperatures between 25°C and 38°C showed that these plants had a larger number of shorter internodes which contained lower sucrose levels than similar

sugarcane plants grown at 23-33°C (Bonnett, Hewitt and Glassop, 2006). High daytime temperatures (above 31°C) may also inhibit flowering, and very high temperatures at anthesis may reduce seed set. However, it has been suggested that these responses to high temperatures may be due to a water stress effect (as discussed in Moore and Nuss, 1987).

Water stress

Sugarcane is relatively drought resistant but water stress results in a reduction of sugar production (FAO, 2004). It is estimated that irrigation can add 3 t sugar per ha, a figure modelled on an average irrigation of 500 mm (Meyer, 1997). Sett germination (sprouting) does not occur in dry soil (Smit, 2011). Sugarcane flowering is also reduced by water stress (Moore and Nuss, 1987), with watered crops showing a greater number of panicles and a higher percentage of plants flowering (Berding, 1995).

Other abiotic stresses

Waterlogging

Sugarcane plants can withstand short periods of flooding (FAO, 2004). After four days, the growing point of the sugarcane plant will die, but it may continue to grow from side shoots once the water has receded (BSES Ltd., 2012d). Generally, yield loss will be 15-20% after 5 days submergence, 30-60% yield loss after 10 days and 37-100% after 15 days, but this depends on the height of the stalks, with younger cane being more affected than those at 2.5 m tall (BSES Ltd., 2012d). However, a pot study in Florida showed that some sugarcane varieties were able to sustain growth during short periods of flooding (Glaz, Morris and Daroub, 2004). Prolonged periods of waterlogging will result in a decline in sugar content (FAO, 2004). Waterlogging also results in cooler soil temperatures so germination (sprouting) of setts will be slower and losses from disease may be higher (Ridge and Reghenzani, 2000).

Altitude

Sugarcane is grown in a range of altitudes from just above sea level to as high as 3 000 m above sea level (FAO, 2004).

Wind

High winds, especially when combined with heavy rain, can lead to lodging of cane stalks in the field. This leads to problems with harvesting, reduced cane yield and reduced sugar content. In Australia, in northern Queensland, a 15-35% decrease in sugar yields has been recorded in a lodged crop compared to an unaffected crop (Singh et al., 2002; 2000). This may be due to rat damage, suckering, and stalk and stool death following lodging (Inman-Bamber et al., 2008).

Breeding for high, above-ground biomass in modern sugarcane cultivars means the plant is very top heavy and consequently sugarcane is prone to lodging. Plants recover from lodging by curving of the stem to again grow upright. Yield losses observed following lodging may be due to rat damage, suckering, stalk and stool death as well as poor ratooning in the following crop (Inman-Bamber et al., 2008). Lodging also leads to reduced light interception.

Soil pH

Sugarcane prefers a soil pH of 5.0-5.8, although it will tolerate a pH of 4-10 (Fauconnier, 1993).

Salt tolerance

Sugarcane is sensitive to soil salinity. It has been estimated that it will show no reduction of growth in soil with salinity up to 1.1 decisiemens per metre (dS per m) and a 10% growth reduction at 2.2 dS per m (Evans, 2006). Sugarcane production is not economic in areas with soil salinity above 4.0 dS per m (Rozeff, 1995). It has been further estimated that there are 1 million ha globally on which sugarcane is grown which are affected by salinity (Hunsigi, 1993) In Pakistan, it has been estimated that 6.3 Mha out of a total land area of 79.6 Mha is salt-affected (Hussain et al., 2004a) and in 1994 this led to significant yield losses (Wahid, Rao and Rasul, 1997). Salinity problems have also been experienced in cane growing areas of south Texas (United States) (Gerard, 1978), the Haft Teppeh region in Iran (Sund and Clements, 1974) and Australia (Christiansen, 2000). Salinity affects both growth rate and yield of sugarcane, but also the sucrose content of the stalk (Rozeff, 1995). Shoot growth has been shown to reduce, although the severity varies between cultivars (Akhtar et al., 2001b), and root growth may be stimulated by increased salinity (Gerard, 1978). High salinity has been shown to reduce stalk height and weight, due to a reduction in both the number of internodes and the internode length, but not the number of stalks, and may be related to reduced water content (Akhtar et al., 2001a; Lingle et al., 2000). Leaf dry weight and area also decrease with increasing salinity (Plaut, Meinzer and Federman, 2000). Different life stages may have different sensitivities to salinity, with seed germination showing the least sensitivity (Wahid, Rao and Rasul, 1997). In experiments under saline conditions, ratoon crops have shown 2.2-3.7 times greater yield loss compared to plant crops (Bernstein, Francois and Clark, 1966). The addition of potassium and silicon have been shown to help ameliorate the decreases in plant growth and juice quality caused by salinity, and actually have more effect on salt-sensitive genotypes compared to salt-tolerant genotypes (Ashraf et al., 2009).

Aluminium tolerance

High aluminium levels are associated with acid soils, and aluminium toxicity can cause a major reduction in yield in many crops (Delhaize and Ryan, 1995). Sugarcane is relatively tolerant of high aluminium levels, although differences in tolerance have been seen between cultivars (Hetherington, Asher and Blamey, 1986). Cultivars of the *S. officinarum* parent species generally have higher levels of tolerance than the *S. spontaneum* parent species (Landell [1989] as cited in Drummond et al., 2001). In an experiment comparing the aluminium tolerances of sugarcane, navy beans, soybeans and maize (corn), which may be grown in rotation with sugarcane, the sugarcane cultivars showed the greatest tolerance. The concentrations of aluminium which led to a 10% reduction in root growth were up to ten-fold higher for sugarcane than the other crops tested (Hetherington, Asher and Blamey, 1988). Symptoms of toxicity include root stubbing, which leads to susceptibility to water stress and yield loss (Calcino, 1994).

Other metals

Sugarcane has been shown to tolerate up to 100 µM copper in laboratory experiments (Sereno et al., 2007). Tolerance to cadmium is higher, with laboratory experiments showing no toxicity at 500 µM cadmium (the highest concentration tested). Plant damage was seen in other experiments at 2 mM cadmium (Fornazier et al., 2002). The high tolerance to cadmium and the observation that the sugarcane plants can accumulate cadmium have suggested its use in phytoremediation (Sereno et al., 2007).

Biotic interactions

Weeds

Weeds are one of the major problems in sugarcane crops due to wide row spacing, slow germination (sprouting) and initial growth, heavy fertilisation and frequent irrigation (Raskar, 2004). Weeds lead to yield reduction caused by competition or allelopathy and interference with harvesting machinery, which reduces product quality (McMahon, Lawrence and O'Grady, 2000). In India, weeds are reported to cause greater yield loss than all pests (Raskar, 2004). Experiments have shown that herbicides applied at planting time can more than double the yield of an untreated crop (Akhtar and Ahmed, 1999) and weed removal leads to increased yield in ratoons (Singh and Tomar, 2005). In Ethiopia, weeds cause a yield loss of 41-51% (Firehun and Tamado, 2006). In Sudan, cane yields were 40% less in unweeded cane than cane fields in which the weeds had been removed (Ibraham, 1984). Other data have suggested that a single species, such as Bermuda grass (*Cynodon dactylon*) in Louisiana, can account for 32% reduction in sugar yield due to reduced sugarcane stalk numbers and height (Richard and Dalley, 2007). Weeds may also act as a reservoir for plant pathogens or pests. As well as controlling weeds within the crop, it is important to control weeds around the farm to reduce any high protein food, such as weed or grass seeds, which rats need to breed (McMahon, Lawrence and O'Grady, 2000). See below for a discussion of rats as a pest of sugarcane.

There are a number of weeds that infest sugarcane plantations including grasses, broadleaf weeds, vines and sedges. The paragraphs below discuss those weeds that are a major problem worldwide. However, the weed population can vary significantly; for example, surveys in Ethiopia concluded that the weed flora varied depending on soil type, fertiliser application and crop cycle, and from year to year in the same region (Firehun and Tamado, 2006).

Imperata cylindrica (lalang or blady grass) is a perennial species that commonly grows on degraded or burnt-off land in most Australian sugarcane-growing districts (Lazarides, Cowley and Hohnen, 1997). It is also listed as a noxious weed in a number of states in the United States (USDA-NRCS, 2013). It is an alternate host to ratoon stunting disease (RSD) in Pakistan (Jabeen and Ahmed, 2010).

One of the most important and prevalent weeds of sugarcane is sedge nut grass (*Cyperus rotundus*, also known as purple nutsedge), although in wetter areas other sedges also occur (McMahon, Lawrence and O'Grady, 2000). It spreads mainly by tubers, which are produced in very large numbers and are carried in soil and by flood waters. It also reproduces by seed, although apparently only rarely. It withstands cultivation extremely well, and this process rapidly spreads the tubers around and between fields (DPIW-Tas, 2009). The FAO lists this as a weed of sugarcane in Colombia.[11] In two studies in India it was the dominant weed species (Murugan and Kathiresan, 2010; Raskar, 2004), and it was identified as a weed in Ethiopian, South African and Argentinean sugarcane fields (Ferraro, Ghersa and Rivero 2012; Firehun and Tamado, 2006; Leibbrandt, 1997). In Ethiopia, Kenya and South Africa it has been identified as one of the three most serious weeds (Bendixen and Nandihalli, 1987). Its prevalence in sugarcane fields in Louisiana has been increasing due to inadequate control during the fallow period (Etheredge, Griffin and Boudreaux, 2010a; 2010b). Pasture grasses can also be problematic when the land is subsequently used to grow sugarcane (McMahon, Lawrence and O'Grady, 2000).

Broadleaf weeds such as blue top/billygoat weed/tropic ageratum (*Ageratum* spp.) and purslane/pigweed (*Portulaca oleracea*) tend to be less of a problem and can be controlled relatively easily if targeted when the plants are young. Broadleaf weeds tend to be more regional and soil specific (McMahon, Lawrence and O'Grady, 2000). In India, the parasitic plant *Aeginetioa pedunculata* causes crop losses of up to 37% due to reduced stalk growth and juice quality (Ray and Dasgupta, 2006).

Vines have become an increasing problem after the adoption of trash-blanketing, although a thick layer of trash has been shown to inhibit their growth (Fillols and Callow, 2010). They have the potential to grow rapidly and if left uncontrolled can impede the harvesters (McMahon, Lawrence and O'Grady, 2000). The most problematic vines in sugarcane include bindweed (*Convolvulus* spp.), passionvine (*Passiflora* spp.) and morning glory (*Ipomoea* spp.) (McMahon, Lawrence and O'Grady, 2000).

Weeds may be controlled either by herbicide use or by mechanical removal. In some countries this is by hand-hoeing, but animal- or tractor-drawn equipment may also be used (Fauconnier, 1993). In most sugarcane growing countries, herbicides are used to control weeds (Cheavegatti-Gianotto et al., 2011). There are a number of herbicides that can be used to control weeds in sugarcane. These include pre-emergent herbicides such as isoxaflutole, imazapic or a diuron/hexazinone mix (Fillols and Callow, 2010). Herbicides such as 2,4-D amine can be used on broadleaf weeds. Paraquat, a non-selective herbicide, can be used on broadleaf, grassy and other weeds (McMahon, Lawrence and O'Grady, 2000).

Pests and pathogens

Pests and pathogens can have a major impact on sugarcane production worldwide. For example, in Australia the cost of controlling the major pests and diseases of sugarcane was estimated to be AUD 111 million in 1996 (McLeod, McMahon and Allsopp, 1999). This included AUD 14 million in lost production and control costs for pests, and AUD 97.4 million in loss and control for diseases (McLeod, McMahon and Allsopp, 1999). This is low compared to other countries, where it can be 10-15% of the crop (as quoted in Plant Health Australia, 2009).

The distribution of sugarcane pests appears to be more specific to a particular country or region, whereas diseases are more ubiquitous across the international sugarcane industry, although the impact of diseases may vary between countries. The major pests and pathogens of international relevance to the sugarcane industry are discussed below.

Pests

Invertebrate pests

There are many invertebrate pests of sugarcane and some insects such as plant hoppers (*Perkinsiella saccharicida*) are also known vectors of diseases (Allsopp, Cox and Nutt, 2002; Croft, Magarey and Whittle, 2000). The impact of invertebrate pests can be large, or be widespread without causing large losses; for example, in China, borers and soil-borne pests were found in 60% of sugarcane plantations but only caused 0.5% loss in sugar content (Chen and Yuan, 2010).

Annex 2.A1 gives an overview of these invertebrate pests.

Plant parasitic nematodes are an important factor in the worldwide decline in sugarcane production (Cadet and Spaull, 2003). A large number of species have been identified from sugarcane fields, with one study in Pakistan identifying 25 different

species from newly planted sugarcane crops (Qureshi et al., 2002) and a Kenyan study identifying 14 different genera from sugarcane fields (Chirchir et al., 2011). The impact of nematodes varies between countries, but has been estimated to cause annual yield losses of between 0.2% in Australia, through >5% in South Africa, to 14% in Burkina Faso (Magarey, 1996). However, other estimates of yield losses have suggested that they may cause a 10% loss in plant crops and a 7% loss in ratoon crops in Australia (Blair and Stirling, 2007).

Nematodes also affect the longevity of the crop, with high levels of nematode damage reducing the number of times a field can be economically ratooned (Cadet and Spaull, 2003). The main tools for control are crop management practices such as crop rotation and mulching, but nematicides may also be employed in some countries (Cadet and Spaull, 2003). In Brazil, application of nematicides can increase crop productivity by up to 30% (Copersucar as cited in Cheavegatti-Gianotto et al., 2011) and experiments using nematicides in South Africa showed an 85% increase in yield over untreated fields for some sites with large *Meloidogyne* populations (Cadet and Spaull, 2003). There are also varying amounts of resistance between different sugarcane varieties to attack by nematodes (Chirchir et al., 2011). It has been suggested that the practice of hilling-up used in Australia may reduce nematode damage on ratoon crops due to a larger, below-ground stool (Berry, Spaull and Cadet, 2007). Pre-trashing with stubble retention altered the proportion of nematodes, with an increase in less pathogenic nematodes and therefore reduced crop damage (Berry, Spaull and Cadet, 2007).

Borers are a major pest in sugarcane worldwide, with stalk borers, shoot borers and internode borers having different impacts in different regions of the world. It has been estimated that stem borers account for 10% of world yield losses in sugarcane (Fauconnier, 1993). The moths lay eggs on young leaves and the larvae burrow into the stem, emerging as adults. In young plants, the inner whorl of leaves can be killed resulting in "dead heart", whereas in older plants the tops may die (Capinera, 2010). This leads to a reduction in sucrose content, reduced tillers and provides entry points for diseases (Purseglove, 1972). In South Africa, the stalk-boring pyralid moth *Eldana saccharina* is highly damaging, with economic impacts of this pest in the order of ZAR 60 million/year (Snyman et al., 2008a). A survey in 2006/07 suggested that 40% of fields were affected, with infection rates varying between <10 to >90% between mill areas (Van den Berg et al., 2008).

In India, the borers *Chilo infuscatellus* (shoot borer), *Chilo sacchariphagus* (internode borer) and *Scripophagua excerptalis* (early shoot borer) are major insect pests of sugarcane (Kalunke et al., 2009). The shoot borers (*Chilo* spp.) are major pests in Asia and Africa (Berding, Hogarth and Cox, 2004) The shoot borer (*Chilo infuscatellus*) attacks the crop early in the season (Arvinth et al., 2010) and some control by the parasitoid *Sturmiopsis inferens* has been reported (Srikanth et al., 2009). The sugarcane stem borer *Diatraea saccharalis* has been described as the most important pest of sugarcane (Bennett [1977] as cited by Arencibia et al., 1997) and inflicts severe losses in Brazil (Braga et al., 2003) and Louisiana (Beuzelin et al., 2011). A second *Diatraea* spp., *D. flavipennella*, is also important in Brazil, as is the giant sugarcane borer (*Telchin licus*) (Cheavegatti-Gianotto et al., 2011). *Diatraea* spp. are controlled by release of the parasitoids *Cotesia flavipes* and *Trichogramma galloi* or by chemical sprays, but these are not effective against the giant sugarcane borer (Cheavegatti-Gianotto et al., 2011). Contact insecticides are not effective as the borers are inside the stems (Kalunke et al., 2009). Sugarcane cultivars differ in their resistance to borers (White, 1993).

Sugarcane thrips *Fulmekiola serrata* are widespread in many areas including Asia, Barbados, Madagascar, Mauritius, Réunion, Trinidad and Tobago, and the Bolivarian Republic of Venezuela (summarised in Way et al., 2006). In South Africa, they were first detected in 2004 (Way et al., 2006) and many fields are affected, although the extent of the damage caused is not known (Van den Berg et al., 2008). In South African field trials, some fields showed yield losses of 18-27 t cane per ha (Way et al., 2010). They are thought to have been transferred to South Africa from Mauritius by wind, or via infected planting material (Way et al., 2006). The thrips cause leaf necrosis due to feeding and in young cane leaf tips can become tied together, or brown and wither (Way et al., 2006).

Cane grubs (melolonthine white grubs, larvae of the endemic melolonthine beetle) are major pests affecting the sugarcane industry in some countries. They destroy the roots of the sugarcane plants, preventing water and nutrient uptake and causing lodging (Allsopp, Samson and Chandler, 2000). In Australia, there are 19 native species of cane grub, which cause significant damage in cane fields in different regions, with the greyback canegrub (*Dermolepida albohirtum*) showing the most widespread damage (Robertson et al., 1995). This was estimated to cause a crop loss of 1 million t of cane in the 2000-01 season (Chandler and Tucker, 2010). In Florida, the white grub (*Ligyrus subtropicus*) has been estimated to cause sugarcane yield reduction of 39% (Cherry, 2008).

Several methods can be used for the control of cane grubs (Robertson et al., 1995). In Australia, the application of insecticide or the biological control agent *Metarhizium anisopliae* (a fungus that attacks the larvae) soon after planting controls the species for two to three years. However, in Florida, insecticides and *M. anisopliae* have not shown to be effective in the field so cultural methods such as disking and flooding are used (Cherry, 2008).

In Florida, the corn wireworm (*Melanotus communis*) is a major pest of sugarcane. They are a pest of plant cane and feed on buds and root primordia causing shoot death and also providing an entry-point for disease (Cherry, 2011). One study showed that one wireworm feeding per 1.5 m row of cane leads to 6.2-7.8% stand reduction at 12 weeks, with a larger study showing a 3.8% reduction in yield at harvest (Hall, 1990). In Okinawa and Kagoshima prefectures in Japan, the wireworm known as the sugarcane click beetle larvae (*Melanotus okinawensis*) is a destructive pest (Ohira, 1988; Setokuchi et al. [1990] as cited by Arakaki, Hokama and Yamamura, 2010).

Spittle bugs or frog hoppers (*Mahanarva fimbriolata*) have become a major pest of sugarcane in Brazil following the decrease in cane burning (Korndörfer, Grisoto and Vendramim, 2011). Infestation reduces stalk productivity and in some cases stalk quality (by reducing sugar content and increasing fibre content) (as discussed in Dinardo-Miranda, Pivetta and Vilela Fracasso, 2008). The shorter and thinner stalks have a concomitant yield reduction of up to 16% per ha (de Souza Rossato Jr. et al., 2011).

In Brazil, borers, termites including *Heterotermes tenuis*, migdolus beetle (*Migdolus fryanus*), spittlebugs/froghoppers (*Mahanarva fimbriolata* and *Mahanarva posticata*), sugarcane weevil (*Sphenophorus levis*) and leaf-cutting ants (*Atta* spp. and *Acromyrmex* spp.) are also important pests (Cheavegatti-Gianotto et al., 2011). Brown burrowing bugs (*Scaptocoris castanea*, *S. carvalhoi* and *Atarsocoris brachiariae*) are secondary pests which cause root damage at high infestation levels. Other insect pests of sugarcane which are important in other countries include sugarcane and yellow soldier flies (*Inopus rubriceps* and *Inopus flavus* respectively), wireworms (*Melanotus communis* in Florida (Cherry, 2011), *Agrypnus variabilis*, *Heteroderes* spp. and *Conoderus* spp.,

armyworms including day and night feeding species, as well as loopers (Allsopp, Samson and Chandler, 2000), aphids (e.g. *Melanaphis sacchari* and *Sipha flava* in Louisiana; Akbar et al. [2010]), weevils (e.g. *Metamasius hemiptera sericeus* in West Indies and Florida; Weissling and Giblin-Davis [2010]) and oriental cinch bug (*Cavelerius saccharivorus*) in Japan.

Vertebrate pests

Vertebrate pests including rodents, pigs, birds and large mammals can cause both eating and trampling damage to sugarcane. In some countries rats are a serious pest of sugarcane. They cause yield loss directly by gnawing the cane, but the damage also allows the cane to dry out and provides entry points for bacterial and fungal attack (Dyer, 2005). In addition, rats are known to be carriers of diseases such as the bacterium *Leptospira*, which can result in Leptospirosis disease in humans. Surveys of rodents in sugarcane plantations in Ethiopia identified eight species of rats, with the highest numbers occurring in young plantations (Serekebirhan et al., 2011). Small mammal damage has been seen on up to 4.7% of stalks in the Wonji area of Ethiopia (Serekebirhan et al., 2011). In South America, three species of rat have been implicated in causing damage to sugarcane plantations (Stenseth et al., 2003).

In Australia, during the 1999 and 2000 seasons, ground rats (*Rattus sordidus*) and climbing rats (*Melomys burtoni*) destroyed 825 000 t of sugarcane valued at AUD 25 million (Dyer, 2005). Integrated pest management is now widely employed to discourage and control these economically damaging pests (Smith et al., 2002). Strategies such as controlling crop weeds have been shown to reduce juvenile rat numbers by 50% and reduce crop damage by 60% (Dyer, 2005).

In Pakistan, wild pigs (*Sus scrofa*) are the most important vertebrate pest in sugarcane. They cause damage by knocking over stalks and tearing away the rind to access the soft inner pith. Damage to sugarcane in one district was estimated at 11% of the crop (Brooks et al., 1989). Other vertebrates such as hippopotami (*Hippopotamus amphibius*), warthogs (*Phacochoerus africanus*) and vervet monkeys (*Chlorocebus aethiops*) cause damage to sugarcane crops in Africa (Serekebirhan et al., 2008), wild pigs are important in Australia and Africa, and jackals (*Canis aureus*) cause damage in India (Purseglove, 1972). Warthogs eat lower internodes of cane and also destroy stalks whilst moving through the plantations. Vervet monkeys remove younger cane and carry it to trees to eat (Serekebirhan et al., 2008).

Pathogens

Various biological agents including bacteria, fungi, viruses and phytoplasma cause diseases of sugarcane. Important diseases of sugarcane that have been identified worldwide are listed in Annex 2.A2. Diseases often lead to large yield losses. For example in China, sugarcane smut, ratoon stunting disease (RSD), mosaic and other diseases cause a greater than 20% reduction in production (Chen and Yuan, 2010). In Australia, losses due to disease are AUD 67 million from a gross value for sugarcane of AUD 2 100 million (Chakraborty et al., 1998).

In South Africa, the fungal pathogen *Usilago scitaminea* (causal agent of smut) and the sugarcane mosaic virus (SCMV) were listed as amongst the most important biotic challenges that sugarcane faces along with the insect stalk borer *Eldana saccharina* (Butterfield et al., 2004; Rutherford et al., 2003).

Disease control in sugarcane is based on resistant cultivars and management procedures. Short-term spraying options are available, but their economic viability may not be sustained. Hygiene is important to disease management strategies, particularly for diseases transmitted through cuttings such as RSD and leaf scald. Cutting one infected stalk may lead to significant infection to the next 100 cuttings, which are subsequently cut by the same blade (Croft, Magarey and Whittle, 2000). Machine harvesters can also transmit disease.

Many sugarcane diseases are also managed through the use of disease-free planting material. Hot-water treatments are used to disinfect planting material. In Australia, long hot-water treatment (three hours at 50ºC) is used to control RSD. Soaking in ambient temperature running water for ~40 hours followed by 3 hours at 50°C is used to control leaf scald bacteria. Short hot-water treatment (50ºC for 30 minutes) is used to control chlorotic streak and some insect pests (Croft, Magarey and Whittle, 2000). In Brazil, a shorter, hotter treatment is used for RSD (52ºC for 30 minutes) (Fernandes Jr. et al., 2010).

Predictions have been made on the impact of climate change on the spread and importance of sugarcane diseases. It has been suggested that the major diseases of sugarcane will not be affected by climate change as they are systemic and spread by human intervention; however, for some diseases such as leaf scald the increased severity and frequency of cyclones and storms may allow it to spread more readily (Sanguino, 2008 as discussed in Ghini, Bettiol and Hamada, 2011). Conversely, the predicted reduced soil temperatures in some regions may reduce the range of diseases such as pineapple disease (*Ceratocystis paradoxa*) (Chakraborty et al., 1998).

Bacterial diseases

RSD is probably the most important disease of sugarcane. It is a highly infectious disease caused by *Leifsonia xyli* (formerly named *Clavibacter xyli* subsp *xyli*), which infects vascular tissues of sugarcane. It has been identified in most countries that grow sugarcane. The symptoms are poor growth and stunted shoots, which might not be obvious if most plants in the field are infected. It has been suggested that a 5-15% yield loss can occur without growers realising that they have the disease (Comstock and Gilbert, 2009). The visual symptoms of red-orange dots in the vascular tissues can be seen only when the stalks are cut and sliced (Croft, Magarey and Whittle, 2000). The disease is transmitted by healthy plants coming in contact with diseased plant material or contaminated cutting implements. Yield loss is higher in dry weather and often becomes more severe in subsequent ratoon crops (Frison and Putter, 1993). In Florida, resistant clones have been used to control the disease (Comstock and Gilbert, 2009).

Leaf scald is caused by the bacterium *Xanthomonas albilineans*, which infects the vascular tissues of sugarcane. It is found in many countries and is thought to have originated in the Old World but had spread to Brazil by 1944 and Guyana by 1950 (Purseglove, 1972). However, it is hard to identify and the disease often has a latent period after infection. Leaf scald is characterised by a long white to cream streak on the leaves. Severely infected leaves appear scalded and roll inwards, with the top of the shoots becoming chlorotic. Yield loss occurs through the death of infected cane stalks and poor ratooning (BSES Ltd., 2012c). Leaf scald can spread by windblown rain, plant material and contaminated cutting equipment such as planters and harvesters (Croft, Magarey and Whittle, 2000; Daugrois et al., 2011). Leaf scald can infect many other grasses which are alternate hosts and act as a reservoir for the disease. Extremes of moisture and temperature favour disease transmission. In Australia, resistant cultivars are

used to curb the spread of the disease and susceptible plants are not used in breeding programmes (BSES Ltd., 2012c).

Fungal and oomycete diseases

The two major rusts in sugarcane are orange and brown (previously known as common) sugarcane rusts (Braithwaite et al., 2009). Orange rust is caused by *Puccinia kuehnii* and is not generally as economically important as the common rust, caused by *P. melanocephala*. These are both obligate parasitic fungi spread by windblown spores. The disease symptoms of the two rusts are distinct. Pustules of the orange rust are orange and tend to be grouped in clusters, while those of brown rust are reddish brown and are distributed evenly on leaves. Pustules rupture the leaves and allow water to escape from the plant, leading to moisture stress (Croft, Magarey and Whittle, 2000). Both diseases are most severe in humid environments with temperatures below 25°C (Walker, 1987).

Brown rust appeared in Australia and the Caribbean in the 1970s. Yield loss from brown rust depends on environmental conditions and was estimated to cause an economic loss in Australia of AUD 3.5 million in 1996 (McLeod, McMahon and Allsopp, 1999) and a yield loss of 20-40% in the United States (Raid and Comstock, 2006). In South Africa, brown rust is common in the Midlands area of KwaZulu Natal (Zhou, 2013).

In the 1999-2000 season, sugarcane crops in Australia were affected by an outbreak of orange rust, which severely damaged the most widely grown commercial cultivar, Q124 (Croft, Magarey and Whittle, 2000). Orange rust was identified from 2007-09 in Mexico, Florida and Central America (Chavarría et al., 2009; Comstock et al., 2008; Flores et al., 2009) and recently in Brazil (Cheavegatti-Gianotto et al., 2011). These diseases are usually controlled by the use of resistant cultivars (Berding, Hogarth and Cox, 2004), although some resistant cultivars have been overcome, presumably due to rust variants (Raid and Comstock, 2006). Yield losses occur due to reduction in leaf photosynthetic components (Zhao et al., 2011).

Sugarcane smut, caused by *Ustilago. scitaminea*, is a serious disease of sugarcane that can reduce yields by 30-100% (BSES Ltd., 2012e). Infection occurs through the sugarcane buds from windblown spores (Walker, 1987). The disease causes severe stunting and multiple thin stalks. It is characterised by black, whip-like structures that form at the growing points of sugarcane plants (Croft, Magarey and Whittle, 2000) (Figure 2.7).

These whips replace the spindle leaves and are formed in the shoots developing from infected cane cuttings (Frison and Putter, 1993). The whips break open to release the mature spores which are spread by wind (BSES Ltd., 2012e). Smut was confined to Asia and southern Africa until the 1970s when it spread to other countries, reaching Australia in 1998, and now only Fiji and Papua New Guinea do not have the disease (Berding, Hogarth and Cox, 2004). In South Africa, smut is more common in northern irrigated areas with losses varying depending on the variety, crop stage and growing conditions (Van den Berg et al., 2008).

In Australia, the spread and occurrence of the disease is being controlled through planting resistant cultivars, using uninfected seed canes and removing infected crops (BSES Ltd., 2012b). In South Africa, the disease is partly controlled by a compulsory plough-out if greater than 10% of the crop is affected (Van den Berg et al., 2008). In Brazil, pre-plant fungicide treatment and rogueing of infected plants are used to control smut (Cheavegatti-Gianotto et al., 2011).

Figure 2.7. **Smut on *Saccharum* spp. hybrid in Bundaberg**

Source: Courtesy staff at OGTR, taken in 2010.

Other fungal diseases of sugarcane are minor (see Annex 2.A2) and cause less impact on yield.

Viral diseases

Sugarcane can be affected by a number of viral diseases (see Annex 2.A2).

Chlorotic streak is thought to be caused by a virus. The disease occurs in many countries, especially in wet and poorly drained fields (Croft, Magarey and Whittle, 2000). The symptoms are yellow to white streaks on the leaf, midrib and leaf sheath. Older streaks change to yellow and are more visible than younger streaks. This is followed by the appearance of chlorosis in the middle of the leaves. Internal vascular bundle tissues may be reddish in colour (Croft, Magarey and Whittle, 2000). The disease is transmitted by soil water and diseased seed cane. In Australia, a lower incidence of the disease is generally found in drier areas (Croft, Magarey and Whittle, 2000). Yield losses may be up to 40%, with waterlogging compounding the losses. Ratooning may also be poor (BSES Ltd., 2012a).

Fiji leaf gall (previously called Fiji leaf disease) is caused by Fiji disease virus (FDV) and can lead to stunting and death of infected plants (Ridley et al., 2006). The initial symptoms are whitish galls raised on the underside of the leaf blade and midrib. Galls are produced due to the disorder of cell proliferation in the phloem and xylem. Galls can vary from white to green and the surface is usually smooth. When the gall is old, the epidermis may be ruptured and appear brown. At an advanced stage of infection, stem development slows down. Successive leaves become smaller and stiffer with the whole top part of the stem developing a fan-like appearance (Croft, Magarey and Whittle, 2000). Fiji disease can be transmitted by infected cuttings and plant hoppers (*Perkinsiella saccharicidae*) are a known vector for the disease. The disease originated in Fiji and has spread to Australia and Madagascar (Berding, Hogarth and Cox, 2004; Walker, 1987). Significant yield loss was recorded in the 1970s in Australia (Croft, Magarey and Whittle, 2000), but due to the intensive management programme put in place, there have been no reports of disease incidence since the 1980s.

Worldwide, sugarcane mosaic is caused by a number of potyviruses, such as SCMV.

The mosaic symptom pattern appears in young growing leaves. Once the leaves are older, infected leaves may appear relatively normal as the mosaic becomes green. Aphids transmit the disease, as can seed produced by infected cane. Mosaic is a serious problem in sub-tropical countries such as Argentina, Pakistan, South Africa and in southern Brazil and Louisiana (Butterfield et al., 2004; Walker, 1987). Currently in Australia, only the SCMV strain A is present, which is a mild form of the virus (BSES Ltd., 2012f). However, yield loss caused by sugarcane mosaic was 40% in some fields in Australia (Croft, Magarey and Whittle, 2000). Another virus, sugarcane streak mosaic virus (SCSMV), which produces similar symptoms, has been identified in Indonesia (Damayanti and Putra, 2011).

Sugarcane yellow leaf virus (ScYLV) causes yellowing of leaves and in severe infections the plant growth is stunted (Gilbert et al., 2009). It is caused by a luteovirus which is transmitted by aphids (Scagliusi and Lockhart, 2000) or by infected stem pieces. High rates of infection have been reported from sugarcane growing regions of South and Central America as well as in the United States (as reviewed by Gilbert et al., 2009) Thailand (Lehrer, Wu and Komor, 2009; Lehrer, Kusalwong and Komor, 2008), Réunion Island (Rassaby et al., 2003) and northern parts of South Africa (Rutherford, Brune and Nuss, 2004). In Brazil, losses of up to 50% in one variety in cooler regions have been reported (Comstock et al., 1994) and up to 37% yield reduction in Réunion Island (Rassaby et al., 2003).

Phytoplasma diseases

Sugarcane can be affected by a number of diseases caused by phytoplasmas (see Annex 2.A2). Phytoplasmas are small wall-less prokaryotes which infect phloem tissues. In sugarcane they cause a number of diseases including sugarcane white leaf (SCWL), sugarcane grassy shoot (SCGS), sugarcane green grassy shoot (SCGGS), sugarcane yellow leaf syndrome (SCYLS) and Ramu stunt (SCRS). The diseases are transmitted by insect vectors feeding on phloem which include leaf hoppers, plant hoppers and psyllids (Marcone, 2002).

Sugarcane white leaf disease occurs in Asia, is a major disease in Thailand and was confirmed in 2001 in Sri Lanka (Kumarasinghe and Jones, 2001). Infected leaves appear white and are narrower and smaller than uninfected leaves; the plants show stunting and profuse tillering (Marcone, 2002). The disease is spread between plants by a leaf hopper (*Matsumuratettix hiroglyphicus*), which acts as a reservior for the phytoplasma and transmits it transovarially to its offspring (Hanboonsong et al., 2002).

Sugarcane grassy shoot is a major disease in India, but also occurs in Bangladesh, Malaysia, Nepal, Pakistan, Sri Lanka and Sudan (reviewed in Marcone, 2002). Disease symptoms include the formation of many overcrowded, thin, soft-textured tillers with chlorotic or yellow leaves. After ratooning, the infected crop resembles a field of grass due to the short tillers (Marcone, 2002). A similar disease has been observed in Thailand, but without the associated leaf coloration, named sugarcane green grassy shoot (Marcone, 2002).

Other biotic interactions

Sugarcane may have symbiotic relationships with a number of bacteria that fix nitrogen (de Carvalho, Gomes Ferreira and Hemerly, 2011).

In Brazil, sugarcane is grown with low nitrogen inputs (50 kg per ha) compared to some other countries, which use >200 kg per ha (Boddey et al., 1991). Until recently, cane was burnt before harvesting in Brazil (see above), so little nitrogen was returned to the field. This low level of nitrogen fertiliser has led to the suggestion that some cultivars of sugarcane can obtain nitrogen via biological nitrogen fixation (BNF). The occurrence of BNF has been suggested in several pot studies where some cultivars of sugarcane have thrived for several generations without the addition of nitrogen (Boddey et al., 1991; Urquiaga, Cruz and Boddey, 1992). Differences were seen between plant genotypes, but it was estimated that BNF could account for 25-60% of the nitrogen assimilated in one study (Boddey et al., 2001) and up to 70% in another study (Urquiaga, Cruz and Boddey, 1992). The organisms responsible for this have not been unequivocally determined. Studies have focused on endophytic bacteria such as *Gluconacetobacter diazotrophicus* (previously called *Acetobacter diazotrophicus*); however, these bacteria were not shown to be producing nitrogenase *in planta* (James et al., 2001). Despite this, a study using *G. diazotrophicus*-inoculated plants found large increases in nitrogen fixation under nitrogen-deficient conditions. This nitrogen fixation did not occur after inoculation with a mutated nitrogenase deficient form of the bacterium (Sevilla et al., 2001).

G. diazotrophicus may also play a role in defence against sugarcane pathogens. It inhibited *in vitro* growth of *Colletotrichum falcatum* (red-rot) (Muthukumarasamy, Revathi and Vadivelu, 2000) and *Xanthomonas albilineans* (leaf scald) (Blanco et al., 2005; Piñón et al., 2002). Additionally, *G. diazotrophicus*-inoculated sugarcane stems were resistant to infection by *X. albilineans* (Arencibia et al., 2006). Inoculation with *G. diazotrophicus* also improved sett germination (sprouting), tiller number and plant height (Suman et al., 2005). There is also some evidence that it may promote sugarcane growth by production of a growth-promoting factor (Sevilla et al., 2001), such as auxin (IAA; indole-3-acetic acid) or by solubilisation of mineral nutrients (as reviewed in Saravanan et al., 2008).

Other bacterial species have been isolated from sugarcane that may play a role in nitrogen fixation including *Agrobacterium diazotrophicus* (Xing et al., 2006), *Herbaspirillum* spp. (Reis, Lee and Kennedy, 2007), *Azospirillum* spp. (Baldani et al., 1997), *Bradyrhizobium* spp. and *Azorhizobium caulinodans* (Thaweenut et al., 2011) and *Burkholderia vietnamiensis* (Govindarajan et al., 2006). Experiments have shown that co-inoculation of *G. diazotrophicus* and *Herbaspirillum* spp. gave enhanced sugarcane biomass compared to inoculation with either the single species, or to uninoculated controls (Muthukumarasamy et al., 2006). Inoculation with an endophyic *Pantoea agglomerans* strain isolated from eucalypts also showed growth promotion in glasshouse trials (Quecine et al., 2012). Field surveys in Brazil, Japan and the Philippines suggested that up to 70% of plant nitrogen was from BNF (Boddey et al., 2001; Yoneyama et al., 1997). Newly developed sugarcane farms showed lower amounts of BNF than some of the established farms (Yoneyama et al., 1997). However, a field-based experiment and surveys of sugarcane fields in Australia showed no evidence of BNF as a source of nitrogen (Biggs et al., 2000). Similarly in South Africa, BNF fixation was not shown to contribute to the available nitrogen (Hoefsloot et al., 2005).

Vesicular-arbuscular mycorrhizal fungi (VAM) have been found in sugarcane fields in association with sugarcane roots. These fungi are known to colonise plant roots and may supply the plant with mineral nutrients, especially phosphorous. Pot experiments, using soil and mycorrhizal spores from cane fields, showed that the addition of VAM increased the yield of soybean and maize (corn) plants. However, no effects have been seen on sugarcane growth from addition of the VAM *Glomus clarum* at various

phosphorus levels in pot experiments (Kelly et al., 2005; 2001). Similar experiments in wheat have shown that although there is no increased yield following root colonisation with VAM, 50% of the phosphorus in the plants had been absorbed via the VAM (Li et al., 2006). Field experiments in South Africa have observed a correlation between soils with high VAM and improved nutrient levels in sugarcane plants (Jamal et al., 2004). In Pakistan, VAM colonisation has also been correlated with reduced severity of red rot (*Colletotrichum. falcatum*) disease (Nasim et al., 2008).

Weediness

Weeds are plants that spread and persist outside their natural geographic range or intended growing areas such as farms or gardens. Weediness is often correlated with weediness of the plant, or a close relative, elsewhere in the world (Groves, Boden and Lonsdale, 2005; Panetta, 1993; Pheloung, Williams and Halloy, 1999). The likelihood of weediness is increased by repeated intentional introductions of plants outside their natural geographic range that increase the opportunity for plants to establish and spread into new environments (e.g. escapes of commonly used garden plants) (Groves, Boden and Lonsdale, 2005).

Modern *Saccharum* spp. hybrid cultivars do not possess many of the attributes commonly associated with problematic weeds such as seed dormancy, persistence in soil seed banks, germination under adverse environmental conditions and a short life cycle (Baker, 1974; Keeler, 1989; Keeler, Turner and Bolick, 1996).

Weediness status on a global scale

An extensive compilation of the world's weed flora is produced by Randall (2002). Most of the information contained in this book has been sourced from Australia and North America, but also includes numerous naturalised floras from many other countries. Randall (2002) lists 12 species of *Saccharum* which have been identified as having a documented weedy history. However, due to species reclassifications, many of these species are now known by alternative names and are no longer in the *Saccharum* genus as described in Table 2.1. The sugarcane parent species, *S. officinarum*, is listed as naturalised, introduced, a casual alien, an economic weed and a quarantine weed in some countries, but has not been recorded as a major weed (Berville et al., 2005; Holm et al., 1997; Lazarides, Cowley and Hohnen, 1997; USDA, 2013b).

The other sugarcane parent species, *S. spontaneum*, is listed by Randall (2002) as naturalised, introduced, a casual alien, an economic and environmental weed, a noxious weed and a quarantine weed in some countries. *Saccharum spontaneum* is listed as one of the 104 most important world weeds by Holm et al. (1997).

S. spontaneum is native to India and recorded as a weed in 33 countries. It has adapted to diverse environments throughout the world, ranging from tropical to sub-tropical regions, most commonly found in central and south-eastern Asia (Holm et al., 1997). *Saccharum spontaneum* is a serious agricultural weed in India, Indonesia, the Philippines and Thailand where it competes vigorously on disturbed sites (Holm et al., 1997). It occurs in wastelands, fallow fields, marshes, on banks of streams and ponds, on sand dunes, along railroads and highways, and in or around agricultural fields. Pure stands of *S. spontaneum* can be found in poor agricultural soils, degraded by fire and overuse (Hammond, 1999; Holm et al., 1997). It is present in Central and South America, Puerto Rico, Florida and Hawaii. In the Panama Canal watershed, it dominates land that is not under cultivation (Hammond, 1999). It is recorded as a noxious

weed in the United States (USDA-NRCS, 2013). Naturalised populations of *S. spontaneum* have been recorded at several locations in Queensland and the Northern Territory in Australia (Bonnett et al., 2008; Magarey et al., 2007), some which have been deliberately planted.

The hybrid of these two species grown as cultivated sugarcane has not been recorded as a major weed (Berville et al., 2005; Holm et al., 1997; USDA, 2013b).

In both Australia and Brazil, sugarcane has been reported as occurring almost exclusively in managed cultivation. In sugarcane growing districts, transient sugarcane plants may occur around fields, but there is no indication that these form self-perpetuating populations (Bonnett et al., 2007; Cheavegatti-Gianotto et al., 2011). Thus, sugarcane does not appear to be a problem as a volunteer weed (Berville et al., 2005).

Saccharum spp. hybrids are not generally recognised as weeds. They have lost many of the critical weedy attributes such as profuse tillering, adaptability to biotic stresses and resistance to pests and diseases that were present in the parental species from which the cultivated sugarcane hybrids were derived. Setts need adequate soil fertility, soil moisture and temperature for germination (sprouting) (Smit, 2011). As discussed earlier in this chapter, most of the cultivated cultivars exhibit low fertility of both pollen and ovules, so flowers in commercial fields rarely set seed (James, 2004). However, data from Bonnett et al. (2008) and Cheavegatti-Gianotto et al. (2011) suggest that viable seed production does occur at low levels in commercial fields in both Australia and Brazil. The literature also suggests that sugarcane seeds need optimum conditions for germination and survival of the resulting seedlings. These conditions may only occur sporadically in natural ecosystems, thus limiting the spread and persistence of sugarcane.

Control measures

Sugarcane plants can be killed by ploughing out the stools and then treating with herbicide (glyphosate) (Willcox, Garside and Braunack, 2000). However, minimum tillage practices often result in inadequate eradication of the old crop (Leibbrandt, 1993). The efficacy of glyphosate for killing sugarcane is affected by various factors, such as cane being in active growth, cane cultivars, soil type and stage of cane growth (Turner, 1980). Sugarcane grown in light soils is more susceptible to herbicide treatment than that grown on heavy soils. The plant is killed more easily when the height of the leaf canopy is between 0.4-0.75 m compared with older cane that has produced stalks (Turner, 1980). Glyphosate is ineffective on recently cut ratoons until germination (sprouting) of buds is completed and tillering is advanced (Chedzey and Findlay, 1985). Rain may also affect the efficacy of herbicide, so it is more effective when used during the dry season (Owende et al., 1995). Research has shown that slashing of cane suppresses apical dominance and generally enhances chemical cane killing action on the regrowth. In addition, considerable improvement of eradication was also obtained when a mechanical under-cutter was used to shear the roots following herbicide application (Leibbrandt, 1993).

Hybridisation

The possibility of genes transferring from *Saccharum* spp. hybrid to other organisms is addressed below. Potentially, genes could be transferred to: cultivated sugarcane populations, other cultivated and naturalised *Saccharum* species, other plant genera and other organisms. For gene transfer beyond the species, potential barriers must be overcome before gene flow can occur successfully. Pre-zygotic barriers include

differences in floral phenology, different pollen vectors and different mating systems, such as stigmatic or stylar incompatibility systems. Post-zygotic barriers include genetic incompatibility at meiosis, selective abortion, lack of hybrid fitness, and sterile or unfit backcross progeny. Even where pre-zygotic and post-zygotic barriers do not exist, physical barriers created by geographic separation can still limit gene transfer to other plants.

Successful gene transfer requires that three criteria are satisfied. The plant populations must: 1) overlap spatially; 2) overlap temporally (including flowering duration within a year and flowering time within a day); and 3) be sufficiently close biologically that the resulting hybrids are fertile, facilitating introgression into a new population (den Nijs, Bartsch and Sweet, 2004).

Intraspecific crossing

The fertility of the commercial sugarcane cultivars is currently poorly understood. This is mainly because seeds are not the primary product of this crop, nor are they used for propagating sugarcane. In addition, asynchronous flowering, both within and between cultivars, makes hybrid seed production in the field ineffective (James, 1980).

As indicated earlier in this chapter, sugarcane flowering is variable in the field and the crop is exclusively vegetatively propagated. Different cultivars of sugarcane produce different amounts of pollen. Self-pollination does occur, which can prevent outcrossing. The frequency of self-pollination can vary widely depending on the parent, with two studies showing 20-100% and 83-100% outcrossing rates in controlled crosses (Hogarth, 1980; McIntyre and Jackson, 2001).

No insect or animal vectors for sugarcane pollen are known. Pollen viability is low and of short duration under natural environmental conditions (Moore, 1976). Even under artificial conditions, storage of sugarcane pollen is difficult and has been the subject of intensive investigations by sugarcane breeders, where the aim is to store valuable pollen. Little data are available on sugarcane pollen dispersal. Information from breeding work in which plants were isolated by 20 m resulted in 3% and 50% of the offspring respectively being the result of out-crossing (Skinner, 1959). From this work, it was suggested that to prevent contamination of controlled crosses, plants should be isolated by 100 m in open forest, or 300 m in the open (Skinner, 1959).

Flowering and viable pollen production are both temperature dependent, which impacts on the degree of crossing expected in different areas.

Natural interspecific and intergenic crossing

Sugarcane is closely related to the genera *Erianthus*, *Narenga*, *Miscanthus* and *Sclerostachya*. These genera and *Saccharum* are collectively known as the *Saccharum* complex and are expected to be sexually compatible at some levels (Bull and Glasziou, 1979; Daniels and Roach, 1987; Grassl, 1980). There are also reports of sugarcane crossing under controlled conditions with species outside of the *Saccharum* complex (discussed below).

Natural interspecific crossing

As discussed above there is likely to be sexual compatibility between *Saccharum* spp. These species are indigenous to different regions of the world, for example *S. barberi* is native to India and *S. sinense* to China (Sreenivasan et al., 1987). *Saccharum robustum* is

indigenous to Papua New Guinea and adjacent islands of Melanesia (Sreenivasan et al., 1987). *Saccharum spontaneum* has a very wide distribution, extending from Afghanistan in the west to the Malay Peninsula, Chinese Taipei and the South Pacific Islands in the east. The final *Saccharum* species, *S. edule*, is restricted to Papua New Guinea and neighbouring islands, but is unable to reproduce sexually due to the immature unopened (aborted) inflorescence (Nair and Ratnambal, 1970).

Many of these species may also be found elsewhere in the world. Some of these species are maintained within sugarcane research stations as germplasm stocks and have been used in breeding programmes to produce new cultivars. In many countries they are likely to be close to areas in which *Saccharum* spp. hybrid is cultivated. However, no published data have been found on the natural occurrence of interspecific hybrids of modern cultivars.

Natural intergenic crossing

As indicated above, the genera *Erianthus*, *Imperata*, *Narenga*, *Miscanthus* and *Sclerostachya* are expected to be sexually compatible at some levels with sugarcane (Bull and Glasziou, 1979). However, in order to cross naturally with the *Saccharum* spp. hybrid the two species need to be located in close proximity and flower at the same time.

Erianthus spp. are distributed discontinuously in Asia, America, the Mediterranean, and the Polynesian islands (Sreenivasan et al., 1987). *Erianthus rockii* is a wild species originating in the Yunnan, Sichuan and Tibetan regions of China (Aitken et al., 2007). *E. alopecuroides*, *E. strictus*, *E. contortus*, *E. coarctatus* and *E. giganteus* are all native to North America (Burner and Webster, 1994). *E. arundinaceus* is distributed in Bhutan, India, Indonesia, Laos, Malaysia, Myanmar, Sri Lanka, Thailand, Viet Nam, the Ryukyus in Japan and south China (Anon, 2011; Chen and Phillips, 2006). They may also be present in sugarcane research station germplasm collections for use in sugarcane breeding.

Miscanthus is distributed from India to Japan (Sreenivasan et al., 1987). A number of *Miscanthus* species are sold as garden plants, so may be more widely distributed. Some groups of *S. robustum* are thought to be products of a spontaneous hybridisation event between *S. spontaneum x Miscanthus* hybrids, in areas where both species occur naturally (Sreenivasan et al., 1987).

Imperata spp. have a wide distribution worldwide and have been identified as a weed of cultivation (Sreenivasan et al., 1987). Some species are sold as garden plants so may be more widely distributed.

Narenga porphyrocoma is found widely in north-east India (Janaki-Ammal, 1942). It has been suggested that the wild cane Hitam Rokan, collected in Sumatra, is a naturally occurring hybrid of *Saccharum* and *Narenga* (Janaki-Ammal, 1942). This suggestion was based on morphological similarity to known synthetic hybrids but has not been confirmed by molecular methods.

Sclerostachya fusca is found widely distributed in India from Kashmir to Bengal and Assam and also in the western Ghats (Parthasarathy, 1948).

Other species which have reports of sexual compatibility outside the *Saccharum* complex such as maize (corn) and sorghum are present in countries in which sugarcane is grown.

This suggests that many of these species are present in sugarcane growing areas, so there may be potential for crossing to occur. However, no modern natural hybrids have been recorded and, as discussed in the next section, viable hybrids with these species have only been produced under experimental conditions using large numbers of plants, often with male sterility to prevent self-pollination.

Crossing under experimental conditions

Species in Saccharum *complex*

There is limited data available on crosses between *Saccharum* spp. hybrid and other species. Data are presented on crosses with *Saccharum*, *Erianthus*, *Miscanthus*, *Bambusa*, *Sorghum* and *Imperata*. Information on crosses performed with the parent species, *S. spontaneum* and *S. officinarum* is included in this section as it may be indicative of the potential for successful crossing with the hybrid.

Saccharum

Successful controlled crosses have been obtained using pollen from commercial *Saccharum* spp. hybrid and *S. spontaneum* as the female parent. This required heat emasculation of *S. spontaneum* to reduce self-pollination (Pan et al., 2004). No data are available on natural crossing with fertile parents.

Erianthus

A number of species of *Erianthus* have been used for crossing with sugarcane. There is an early report of a cross between *S. spontaneum* and *E. ravennae*, which produced fertile hybrids, although these were not confirmed by molecular methods (Janaki-Ammal, 1941).

Crosses between *Erianthus arundinaceus* and *Saccharum* spp. hybrid produced putative intergenic hybrids which had characteristics from the male *Erianthus* parent. However, these were shown to be selfed progeny which did not possess isozyme marker bands characteristic of *Erianthus* (Lee et al., 1998). A small number of successful crosses were made between *Saccharum* spp. hybrid and *E. arundinaceus* (Lee, Berding and Bielig, 1993). Of 96 attempted crosses between *E. arundinaceus* and *S. officinarum* or hybrid *Saccharum* spp., 26 were successful producing over 1 000 seedlings. Thirty-seven of the seedlings were identified as genuine hybrids, but only 19 survived, all derived from *S. officinarum* as a female parent and *E. arundinaceus* as a male parent. All of these hybrids had poor vigour, were sterile and showed chromosome elimination (Piperidis et al., 2000). Nonetheless, Cai et al. (2005) have successfully identified a fertile intergeneric cross between *E. arundinaceus* and *S. officinarum* using microsatellite markers and 5S rDNA. Genomic slot blot hybridisation (GSBH) has also been used to confirm hybrids between *S. officinarum* (as the female parent) and *E. arundinaceus* (as the male parent) and to determine that 43% of the F_1 progeny were selfs (Besse et al., 1997). Isozyme electrophoresis, sequence-tagged PCR, RFLP and GISH have also been used to confirm intergenic hybrids of *S. officinarum x E. arundinaceus* (D'Hont et al., 1995).

Crosses have been performed with *E. rockii*. These used *S. officinarum* or *S. officinarum* x *S. spontaneum* as the female parent to produce viable hybrids (Aitken et al., 2007). Seed was tested using DNA markers which confirmed the following crosses: *S. officinarum* with *E. arundinaceus*; *Saccharum* spp. hybrids with

E. arundinaceus; and *Saccharum* spp. with *E. rockii* (Foreman et al., 2007). Similarly, crosses between *Saccharum* spp. hybrids and *E. fulvus*, using the *Saccharum* spp. as the female parent, have produced hybrids, confirmed using sequence-characterised amplified region (SCAR) markers (Wang et al., 2009). Hybrids of *S. officinarum* and *Erianthus procerus* have also been generated (Rajeswari et al., 2009).

Crosses have also been performed using elite sugarcane cultivars as the female parent with North American *Erianthus* spp. *E. alopecuroideum*, *E. contortus* and *E. giganteus*. Seed was produced, but it is not known if the progeny were true hybrids (Burner and Webster, 1994).

Narenga

Crosses have been made between *Narenga porphyrocoma* and *S. spontaneum* (Kandasami, 1961). Analysis of hybrids between *N. porphyrocoma* and *S. officinarum* showed intermediate characteristics and low male and female fertility (Janaki-Ammal, 1942). Hybrids from a cross between *S. officinarum* and *N. porphyrocoma* showed viable pollen in tissue culture, although were not verified by molecular methods (Krishnamurthi, 1993).

Miscanthus

Hybrids have been reported from crosses between *Miscanthidium violaceum* (= *M. flavescens*) and *Saccharum* spp. hybrids (Brett, 1954) and crosses and backcrosses between *Miscanthus sinensis*, *Miscanthus floridulus* and *Saccharum* spp. hybrids (Tai et al., 1991). Other crosses with *Miscanthus* have been verified by *Alu*-PCR, using short interspersed nuclear elements (SINE) (Alix et al., 1999).

Sclerostachya

Parthsarathy (1948) reported a cross between *Saccharum officinarum* and *Sclerostachya fusca*. Further crosses between *Saccharum officinarum* and *Sclerostachya fusca* have also been performed. An F_1 hybrid with these two parents has been described as containing 55 chromosomes, and used in tissue culture to regenerate plantlets (Sreenivasan and Sreenivasan, 1984). Four crosses between *Saccharum spontaneum* and *Sclerostachya fusca* have been described, which produced 79 offspring (Kandasami, 1961).

Species outside *Saccharum* complex

Hybridisation with *Saccharum* has also been attempted with some members of distantly related genera belonging to tribe Andropogoneae, such as *Imperata cylindrica* (blady grass or lalang), *Sorghum* spp. and *Bambusa arundinaceae* (bamboo) (Janaki-Ammal, 1938a; Nair, 1999; Rao et al., 1967; Thomas and Venkatraman, 1930) as well as *Zea mays* (maize) from the tribe Maydeae (Janaki-Ammal, 1941; 1938a). In some of these reports, intergenic hybrids were claimed; however, some could not be accepted as true hybrids (Bonnett et al., 2008; Grassl, 1980). As discussed in Bonnett et al. (2008), altered morphological characters and chromosome numbers can occur in self-pollination and are not in themselves proof of hybrid production.

Maize (corn)

A cross was reported between *Zea mays* and *Saccharum officinarum*, using male sterile sugarcane as the female parent (Janaki-Ammal, 1941; 1938a). This plant was sterile, had 52 chromosomes, was morphologically different from both parents and

resolved from both parents based on cluster analysis of random amplification of polymorphic DNA (RAPD) markers (Janaki-Ammal, Jagathesan and Sreenivasan, 1972; Janaki-Ammal,1941, 1938a; Nair et al., 2006, 2005). Another report suggested that the hybrid embryos of maize and sugarcane aborted during development. This was partially overcome by embryo culture, although all the seedlings died when transferred to soil (Hrishi and Marimuthammal, 1968).

Bamboo

Early crosses of *Bambusa arundinacea* with two *Saccharum* spp. hybrids produced 29 hybrids (Venkatraman, 1937). Histological analysis showed that the hybrids had altered chromosome numbers from the parents, and many of the hybrids were male sterile (Janaki-Ammal, 1938b). A cross of *B. arundinacea* with *S. officinarum* produced two progeny (Raghavan, 1952). However, it has been suggested that neither of these were genuine hybrids (Grassl, 1980; Nair and Ratnambal, 1970). Histological analysis of crosses between *B. arundinacea* and *S. officinarum*, *S. robustum*, *S. spontaneum* or seven *Saccharum* hybrids indicated that with *Saccharum* as a female parent, the hybrid embryos aborted during the early embryogenic stage (Rao et al., 1967). Four mature putative hybrid seeds were obtained from 960 crosses using *B. arundinacea* as a female parent, all with either *S. spontaneum* or *S. robustum* as male parents. These either failed to germinate from seed or produced abnormal seedlings which did not survive (Rao et al., 1967).

Sorghum

Sorghum species have been artificially crossed with *Saccharum* spp. hybrids and *S. officinarum* (Grassl, 1980; Gupta, Harlam and de Wet, 1978; Nair, 1999; Thomas and Venkatraman, 1930). These studies used *Saccharum* spp. as both the female or male parent and often used large numbers of sterile lines. Four hybrids were produced using *S. officinarum* as the male parent (Nair, 1999). One of the sterile hybrids was induced to flower by gamma irradiation of calli, and appeared to be female fertile, although male sterile (Sobhakumar and Nair, 2005).

Generally, the hybrid offspring have been of low vigour and fertility, but backcrossing to both parents has been achieved (Grassl, 1980; Sreenivasan et al., 1987). However, Grassl (1980) recorded that after the fourth to fifth generation of backcrossing to *Sorghum*, the sugarcane chromosomes had been eliminated from the intergeneric hybrids. The initial reports used morphological and cytological characteristics to identify hybrids, but more recent work has used RAPD molecular markers to confirm that the hybrids are genuine (Nair et al., 2006; 2005).

Experiments using different *Sorghum* species have shown that pollen-pistil incompatibility is the major barrier to the production of *Sorghum* hybrids (Hodnett et al., 2005). Consequently, breeding work using a *Sorghum* IAP (inhibition of alien pollen) mutant in which the incompatibility is removed as the female parent has produced a number of hybrids with *Saccharum* spp. The hybrid seed produced needed careful management to avoid either vivipary or lack of germination due to an impenetrable seed coat. The hybrids had varied phenotypes from very poor growth to very vigorous, though two of the vigorous plants were male sterile (Hodnett et al., 2010).

Imperata

There is one report of an experimental cross between *Imperata cylindrica* and a *Saccharum* spp. hybrid producing triploid progeny resembling sugarcane, which could

apparently self-fertilise to produce F_2 progeny (Janaki-Ammal, 1941). However, other authors have suggested that these may not have been true hybrids (Nair and Ratnambal, 1970).

Thus, intergeneric gene transfer involving existing commercial sugarcane hybrids may be possible, by hand-pollination under experimental conditions designed to overcome natural barriers to cross-pollination, but such hybrids have not been observed in the wild.

Health and biosafety

Sugarcane is a well-established agricultural crop with a long history of safe use. Commercial sugarcane is grown as a source of sugar (sucrose) for human food. By-products from sugarcane processing include molasses and bagasse, which are mainly used for industrial purposes such as ethanol production and power generation, but also have minor food and stockfeed uses.

Information on processing of sugarcane and its major products (whole cane, sugar, sugarcane juice, molasses, bagasse), as well as food and feed safety considerations including composition in terms of key food and feed nutrients, anti-nutrients and toxicants, have been summarised by the OECD as part of the series dealing with the safety of novel foods and feeds (OECD, 2011) so will not be included here.

Environmental allergens

Sugarcane pollen is transported by wind and therefore has the potential to act as an airborne allergen. The allergenicity of sugarcane pollen was evaluated in India where 70% of field workers with respiratory disorders showed positive reactions to sugarcane pollen in skin tests (Chakraborty et al., 2001). The authors also tested rice and several other plant species and concluded that sugarcane pollen was the most significant allergenic type. A study in Japan of children known to be sensitive to allergens showed less than 3% reacted to sugarcane pollen in tests (Agata et al., 1994).

Exposure to organic dusts, such as those present in mouldy sugarcane, can cause bagassosis. Bagassosis is an occupational lung disease of the extrinsic allergic alveolitis type and is caused by breathing dusts containing fungal spores and/or thermophilic actinomycetes which grow in stored, mouldy bagasse (Lacey and Crook, 1988). In Australia, bagasse may be stored covered with tarpaulins at the end of the crushing season to be used to fuel the boilers at the beginning of the next season before fresh bagasse is available (Dawson, Scott and Cox, 1996). The stored sugarcane bagasse contains approximately 50% water and 5% sucrose, so is colonised by bacteria, causing it to heat up and create ideal conditions for fungi and thermophilic bacteria such as *Aspergillus fumigatus*, *Thermoactinomyces vulgaris* and *Thermoactinomyces sacchari* (Lacey and Crook, 1988). In India, it is thought that *T. sacchari* and *Saccharopolyspora rectivirgula* are the most likely cause of bagassosis (Khan et al., 1995). Prolonged, repeated exposures can lead to permanent lung damage and scarring, and significant disability (Hur et al., 1994; Phoolchund, 1991). However, a study at two Australian sugar mills did not identify very high levels of airborne bacterial spores and none of the 271 mill workers surveyed showed any symptoms of bagassosis (Dawson, Scott and Cox, 1996). In Puerto Rico, a study showed a four-fold increase in risk of cancer of the oral cavity amongst sugarcane farmers and farm workers, which may be due to exposure to actinomycetes (Coble et al., 2003).

There are no reports in the literature of sugarcane causing allergic reactions in humans through consumption (OECD, 2011).

Notes

1. In some taxonomic classifications, *S. barberi* is classified as a subspecies – *S. officinarum* subsp. *barberi* (Jeswiet) Burkill; other classifications do not distinguish between *S. barberi* and *S. sinense* as separate species (*www.theplantlist.org*) (WCSP, 2013).
2. In some taxonomic classifications, *S. edule* is classified as *Saccharum spontaneum* var. *edule* (Hassk.) K. Schum. & Lauterb (*www.uniprot.org/taxonomy*); other classifications do not distinguish between *S. edule* and *S. robustum* (*www.biodiversitylibrary.org*).
3. This sentence was updated in February 2016.
4. This reference was added in February 2016
5. See: *www.ogtr.gov.au*.
6. See: *http://bch.cbd.int/database/lmo-registry.*
7. See: *www.aphis.usda.gov/brs/status/relday.html.*
8. See: *www.daff.gov.za.*
9. This sentence was updated in February 2016.
10. Calculated assuming 1 850 seeds per g (Rao, 1980).
11. See: *www.fao.org/agriculture/crops/core-themes/theme/biodiversity/weeds/db-countries.*

Annex 2.A1.
Major invertebrate pests of sugarcane and their control

Table 2.A1.1. **Major invertebrate pests of sugarcane and their control**

Common name	Species	Affected plant part	Control
Cane grubs	Many species of beetle larvae including *Antitrogus consanguineus* (Australia), *Dermolepida albohirtum* (Australia) and *Holotrichia serrata* (India)	Roots – significant root damage destabilises stool leading to lodging	Primarily insecticide sprays, biocontrol agents, cultural practices and light traps (Japan)
Cicadas	3 species, nymphs	Roots – sap feeding	No chemical control, plough out and leave bare fallow for a season
Ants	*Aphaenogaster pythia, Atta* spp. and *Acromyrmex* spp.	Roots – weakens stools	No chemical control, plough out
Symphylans	*Hanseniella* spp.	Roots – poor crop establishment	Encourage rapid germination, insecticides
Nematodes	Several genera including *Meloidogyne*, *Pratylenchus* and *Helicotylenchus*	Roots – interfere with water and nutrient absorption	Nematicides, crop management practices, resistant varieties
Wireworms (click beetle larvae)	*Agrypnus variabilis, Heteroderes* spp., sugarcane click beetle (*Melanotus okinawensis*: Japan) and *Melanotus communis* (United States)	Shoots – bore into the buds of setts or the growing point	Insecticides in plant crops (none for ratoon crops), flooding, sexual pheromone traps (Japan)
Adult beetles	*Heteronychus arator*, *Metanastes vulgivagus*, *Rhyparida* spp. and *Migdolus fryanus*	Shoots – chew into young shoots causing death of the shoot	Plough out and leave bare fallow for a season, insecticides
Spittle bugs/ froghoppers	*Mahanarva fimbriolata* (Brazil), *M. postica*, *Tomaspis saccharina*, *Aeneolamia varia saccharina* and *Deis flavopicta*	Feed on shoots and leaves	Biological control (including *Metarrhizium anisopliae* in Brazil), insecticides
Weevils	*Stenocorynus* spp., *Naupactus leucoloma*, *Sphenophorus levis* and *Metamasius hemiptera sericeus*	Shoots – also bore into setts and ratoons (occurs rarely)	None available
Shoot borers	African stem borer (*Eldana saccharina*: South Africa), Asian spotted stem borer (*Chilo saccharaphagus*: Mauritius, Réunion, Madagascar and Mozambique), early shoot borer (*Chilo infuscatellus*), internode borer (*Chilo sacchariphagus*), top borer (*Scripophagua excerptalis*: India), sugarcane stem borer (*Diatraea saccharalis*: Americas), sugarcane giant borer (*Telchin licus*: Americas), *D. flavipennella, Ephysteris promptella*, guaspur borer (*Bissetia steniellus*: Pakistan), Mexican rice borer (*Eoreuma loftini*: United States) and *Proceras venosatus* (China)	Shoots – chew into young shoots causing death of the shoot	Parasitoids (including *Cotesia flavipes* to control *D. saccharalis* in Brazil), chemical control, sexual pheromone traps (Japan)
Thrips	*Fulmekiola serrata*	Leaf necrosis, young cane tips tied together, brown and wither	None available
Sugarcane weevil borer	*Rhabdoscelus obscurus*	Stem – bore into stems allowing other diseases in	No chemical control, quarantine between growing areas of sugarcane and palms
Termites	Several species including *Heterotermes tenuis* (Brazil)	Stem – hollow out stems	No chemical control, remove dead wood from cane fields, biological control (*Beauveria bassiana* in Brazil)
Locusts	Several species	Leaf and stem – chewing	Cultivation before eggs hatch
Armyworms and loopers	Various species	Leaf and stem – chewing	Plants usually recover from early damage
Planthopper	*Perkinsiella saccharicida*	Leaf and stem – sap feeding, vector for Fiji disease	Fiji disease-resistant cultivars

Table 2.A1.1. **Major invertebrate pests of sugarcane and their control** (*continued*)

Common name	Species	Affected plant part	Control
Mealybug	Many species including *Saccharicoccus sacchari* and *Pseudococcus sacchari*	Leaf and stem – sap feeding	Natural enemies
Aphids	Several species including woolly aphid (*Ceratovacuna lanigera*: Asia) and sugarcane aphid (*Melanaphis sacchari*: United States)	Leaf and stem – sap feeding	Natural enemies
Scale insect	Many species including *Aulacaspis madiunensis* and *Melanaspis glomerata*	Leaf and stem – sap feeding	Disease-free planting material

Annex 2.A2.
Major diseases of sugarcane

Table 2.A2.1. **Major diseases of sugarcane**

Common name	Causal agent	Distribution	Control
Bacterial			
Leaf scald	*Xanthomonas albilineans*	Worldwide	Resistant cultivars
Ratoon stunting disease (RSD)	*Leifsonia xyli* (previously called *Clavibacter xyli* subsp. *xyli*)	Worldwide	Disease-free planting material
Gumnosis	*Xanthomonas vasculorum*	Widespread in windswept regions	
Red stripe (top rot)	*Acidovorax avenae* subsp. *avenae*	Australia, Iran, Japan, Mexico, the United States and Central America	Resistant cultivars, planting dates
Fungal			
Rusts	*Puccinia melanocephala* and *P. kuehnii*	Africa, Asia and Latin America	Resistant cultivars, fungicide
Downy mildew	*Sclerospora sacchari*	Australia, China (People's Republic of), the Philippines and Chinese Taipei	
Red rot	*Glomerella tucumanensis* (previously called *Colletotrichum falcatum*)	Worldwide, wet and cold regions	Resistant cultivars
Yellow spot	*Mycovellosiella koepkei* (previously called *Cercospora koepkei*)	Widespread in South and East Asia, also in Australia and Oceania. In Africa: Ghana, Mauritius, Réunion Island, South Africa, Tanzania and Uganda	Resistant cultivars
Pachymetra root rot	*Pachymetra chaunorhiza*	Australia	Resistant cultivars
Sugarcane smut	*Ustilago scitaminea*	Worldwide (except some islands)	Resistant cultivars, hot water treatment
Pineapple disease	*Ceratocytis paradoxa*	Worldwide	Fungicide applied to setts
Eye spot	*Bipolaris sacchari*	Many sugarcane growing regions	Resistant cultivars
Pokkah boeng ("tangle top")	*Fusarium monoliforme* (*Gibberella fujikuroi*) and *F. subglutinans* (*G. subglutinans*)	Most sugarcane growing regions	Plants usually recover without need for disease control
Viral			
Chlorotic streak	Unknown, probably virus		Disease-free planting material, good drainage
Fiji disease	Fiji disease phytoreovirus (FDV)	Australia, Fiji, Madagascar, the Philippines and Thailand	Resistant cultivars
Mosaic diseases	Potyviruses: sugarcane mosaic virus (SCMV), *Sorghum* mosaic virus (SrMV), maize dwarf mosaic virus (MDMV), Johnson grass mosaic virus (TGMV) and striate mosaic associated virus	More serious in temperate regions. Not in Guyana, Mauritius and West Africa	Disease-free planting material and resistant cultivars
ScYLV	Sugarcane yellow leaf virus	Australia, Brazil, Colombia, Guadeloupe, Hawaii, Malawi, Mauritius, Réunion Island, South Africa and the United States	Disease-free planting material and resistant cultivars
Phytoplasma			
Sugarcane white leaf		Thailand	Control of insect vectors
Sugarcane grassy shoot		India	Control of insect vectors
Sugarcane yellow leaf syndrome		Australia, Cuba, Mauritius, South Africa and United States	Control of insect vectors
Ramu stunt		Papua New Guinea	Resistant cultivars

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Chapter 3.

Cassava (*Manihot esculenta*)

*This chapter deals with the biology of cassava (*Manihot esculenta*). It contains information for use during the risk/safety regulatory assessment of genetically engineered varieties intended to be grown in the environment (biosafety). It includes elements of taxonomy, centres of origin and distribution, crop production and cultivation practices, morphological characters, reproductive biology, genetics, hybridisation and introgression, interactions with other organisms, pests and pathogens, and biotechnological developments.*

This chapter was prepared by the OECD Working Group on the Harmonisation of Regulatory Oversight in Biotechnology, with Brazil as the lead country and the collaboration of two observer organisations: the African Biosafety Network of Expertise of the New Partnership for Africa's Development (NEPAD-ABNE) and the ILSI Research Foundation, Center for Environmental Risk Assessment (CERA). It was initially issued in June 2014. Production data have been updated based on FAOSTAT, and Figure 3.3 has been added.

Species or taxonomic group

Classification and nomenclature

The scientific name of cassava is *Manihot esculenta* Crantz (ITIS, 2012), synonym *Manihot utilissima* Pohl (Nassar and Ortiz, 2006). Cassava is a member of the spurge family, and its taxonomic hierarchy is:

Order Malpighiales

Family Euphorbiaceae

Genus *Manihot*

Species *Manihot esculenta* Crantz

Subspecies *M. esculenta* Crantz ssp. *esculenta*

M. esculenta Crantz ssp. *flabellifolia* (Pohl) Cifferi

M. esculenta Crantz ssp. *peruviana* (Müeller) Allem (Allem, 2002)

Three subspecies of cassava have been recognised: *Manihot esculenta* ssp. *esculenta* is the cultivated strain, and *M. esculenta* ssp. *flabellifolia* and *M. esculenta* ssp. *peruviana* are wild forms (Allem, 2002; 1999). In this chapter, "cassava" will be used to refer to the cultivated strain, *M. esculenta* ssp. *esculenta*. Common synonyms in other languages are *manioc* (French); *mandioca*, *macaxeira* and *aipim* (Portuguese); *yuca* (Spanish); and *manioca* (Italian).

Approximately 98 species were originally identified in the *Manihot* genus, using morphological and botanical characteristics, and there is one species in a closely related genus, *Manihotoides pauciflora* (Rogers and Appan, 1973; Janick and Byrne, 1984). As modern molecular genetics tools are used in the analysis of the genus, the number of true *Manihot* species is expected to decrease (Duputié et al., 2007). In addition, due to the conversion of native habitat to agriculture, and the resultant destruction of wild species, some of the previously identified species may now be extinct in the wild (Nassar, 2000).

The wild subspecies *M. esculenta* ssp. *flabellifolia* and *M. esculenta* ssp. *peruviana*, as well as *M. pruinosa* have been identified as close relatives of cultivated cassava and are interfertile with cassava (Roa et al., 1997; Allem, 1999; Olsen and Schaal, 1999; Andersson and de Vicente, 2010). Several additional species are included in cassava's secondary gene pool (Table 3.1), and experimental crosses are possible with all these species, although F_1 hybrids tend to be sterile (Andersson and de Vicente, 2010).

Description

Cassava typically grows as a perennial shrub, one to five metres in height (Figure 3.1), with palmate leaves bearing three to nine lobes and covered with a shiny, waxy epidermis. The mature plant generally takes one of two forms: either spreading stems or erect stems with various amounts of terminal branching (Janick and Byrne, 1984; Alves, 2002). Species in the genus *Manihot* are generally well adapted to tropical regions, where they take the form of subshrubs to small trees, forming large, woody roots.

Due to the high level of morphological variability among cassava varieties, it is difficult to reliably distinguish individual varieties using only morphological characteristics (Alves, 2002). To an increasing extent, DNA molecular markers are being used to characterise varieties and measure genetic diversity within the species (Fregene

and Puonti-Kaerlas, 2002). Germplasm preservation programmes in numerous countries worldwide have a combined collection of over 20 000 accessions of cassava (Lebot, 2009).

Table 3.1. **Species within the secondary gene pool of cassava**

Species	Origin and distribution
M. carthagenensis ssp. *carthagenensis* (Jacq.) Müll. Arg.	Antilles, Argentina, Bolivia, Colombia, Paraguay, Trinidad and Tobago, Venezuela
M. carthagenensis ssp. *glaziovii* (Müll. Arg.) Allem (*M. glaziovii* Müll. Arg.)	Native to Brazil, cultivated and naturalised elsewhere (Africa, Asia, Pacific Islands)
M. carthagenensis ssp. *hahnii* Allem	Brazil
M. aesculifolia (Kunth) Pohl	Belize, Costa Rica, El Salvador, Guatemala, Mexico, Panama
M. anomala Pohl	Argentina, Bolivia, Brazil, Paraguay, Peru
M. brachyloba Müll. Arg.	Throughout Central and South America (from Nicaragua to Brazil)
M. chlorosticta Standl. & Goldman	Mexico
M. dichotoma Ule	Brazil
M. epruinosa Pax & K. Hoffm.	Brazil
M. gracilis Pohl	Brazil
M. leptophylla Pax & K. Hoffm.	Brazil, Ecuador, Peru
M. pilosa Pohl	Brazil
M. pohlii Wawra	Brazil
M. tripartita (Spreng.) Müll. Arg.	Bolivia, Brazil, Paraguay
M. triphylla Pohl	Brazil

Source: Andersson and de Vicente (2010). Reprinted with the permission of John Hopkins University Press.

Cassava is grown primarily for its enlarged storage roots, which are used for human consumption, following a variety of traditional processing methods including boiling, roasting, processing into flour, and fermentation (Salick, Cellinese and Knapp, 1997; Hillocks, 2002). Although cassava has the lowest protein-to-carbohydrate ratio among major crops (Sayre et al., 2011), it plays an important dietary role in the diets of almost 1 billion people worldwide (Prochnik et al., 2012). In some regions, particularly in Africa and Brazil, the foliage may also be harvested for human consumption and animal feed, providing supplemental dietary protein (Hillocks, 2002). Cassava is also grown for industrial purposes, such as the production of starch and for fermentation into ethanol (El-Sharkawy, 2004; Adelekan, 2010).

Analyses of the susceptibility of crops to the impacts of climate change indicate that cassava may be better suited to survive climatic variations than most major tropical staple crops, which would make it a key food security crop for the future. However, while calculations indicate that cassava has the potential to produce and store more carbohydrate than any other major grain or root crop, it typically fails to reach that potential due to poor-quality planting material, sub-optimum agronomic practices, and disease and insect pests (El-Sharkawy, 2004; Fermont et al., 2009; Jarvis et al., 2012).

The roots and leaves of cassava and other *Manihot* species are known to release hydrogen cyanide (HCN), which can be toxic to humans and animals when consumed, although the incidence of cyanide poisoning is rare (OECD, 2009). Cassava varieties are classified as "bitter" (glucoside content > 100 mg/kg fresh wt) or "sweet" (glucoside content < 100 mg/kg fresh wt) according to their level of HCN production (Alves, 2002; Peroni, Kageyama and Begossi, 2007). Cassava breeding programmes actively select for varieties which produce lower levels of HCN (Janick and Byrne, 1984), but some farmers

favour cassava with a high cyanide content due to the belief that such varieties are more insect and stress resistant and less prone to theft by humans and predation by mammals (Janick and Byrne, 1984; Fregene and Puonti-Kaerlas, 2002; Lebot, 2009). Most traditional processing methods of cassava enable the safe dissipation of any HCN produced by the plant, and industrial processing methods also remove HCN; however, when large amounts of cassava are processed, toxic effluents can be generated (Taylor et al., 2004). The food and feed processing and use of cassava are described in the "Consensus document on compositional considerations for new varieties of cassava" (OECD, 2009).

HCN is released through the hydrolysis of two cyanogenic glycosides, primarily linamarin, with lower levels of lotaustralin, and hydrolysis is initiated by physical disruption of plant tissues. Linamarin is hydrolysed by linamarase to release HCN. Linamarin is contained in the vacuoles of intact plant cells, while linamarase is located in the cell walls. Tissue disruption allows the two compounds to react (Alves, 2002).

Figure 3.1. **Cassava growing in Nigeria**

Source: Courtesy Dr. Ismail Rabbi, International Institute of Tropical Agriculture, Ibadan (IITA).

Geographic distribution, ecosystems and habitats, cultivation and management practices, centres of origin and diversity

Geographic distribution

Thirty countries (18 in Africa, 4 in Latin America and 8 in Asia) are considered to be major global cassava growers, each producing from 1 million tonnes to over 50 million tonnes annually (FAOSTAT, 2014). The top five cassava producing countries are Nigeria, Thailand, Indonesia, Brazil and the Democratic Republic of the Congo. The global production of cassava exceeded 270 million tonnes in 2014, the top ten producers shown in Figure 3.3 having together produced 74% of it (FAOSTAT, 2014). The species in the genus *Manihot* are native to the New World, falling into two distinct groups, one in Central America and the other in South America. Mexico and Brazil have the greatest number of *Manihot* species, and there are several recognised centres of diversity: central Brazil, north-eastern Brazil, western Mato Grosso (Brazil), south-western Mexico and Bolivia (Nassar, 2000).

Cultivation of cassava is largely limited to the tropics, where the annual mean temperature is greater than 18°C (Figure 3.2) (Kawano, 1980). Only a few *Manihot* species (e.g. *M. neusana* and *M. grahamii*) can survive in areas where frost occurs (Nassar and Ortiz, 2006). Cassava can tolerate drought but performs well at annual rainfall of 600-1 500 mm and temperatures of 25-29°C (Nassar and Ortiz, 2006). It is grown throughout all tropical regions of the world between latitudes 30°N and 30°S and at up to 2 000 m altitude, where day length is 10-12 hours (Alves, 2002). After centuries of cultivation and landrace selection, there are many varieties developed for specific landscapes, elevations, temperatures and soil types (Salick, Cellinese and Knapp, 1997; El-Sharkawy, 2004).

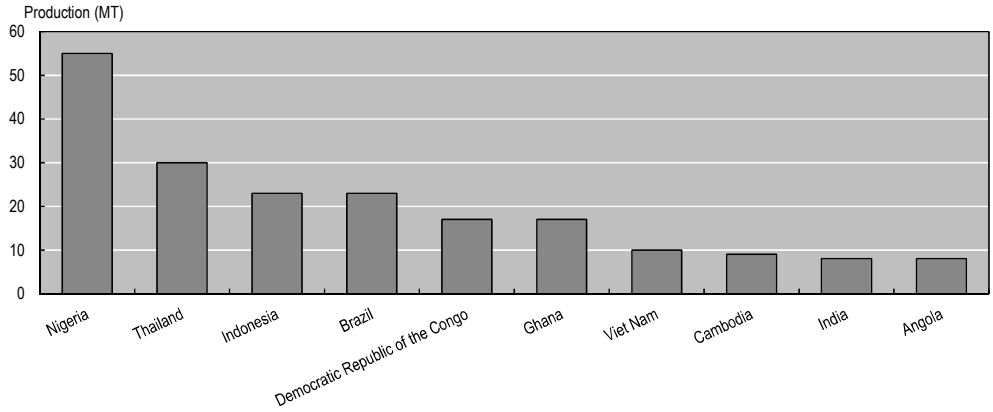
M. glaziovii (*M. carthagenensis* ssp. *glaziovii*) was brought to Africa as a source of rubber. It is the only species within *Manihot* that is known to have naturalised in Africa.

Figure 3.2. **Distribution map showing the widespread cultivation of cassava**

Note: The dark dots represent cassava cultivation points with over 1 000 ha.

Source: CIAT (2002).

Figure 3.3. **Cassava production by top ten producing countries in 2014**



Source: Compiled from FAOSTAT (2014).

Ecosystems and habitats where cassava natively occurs and has been naturalised

Cassava itself does not grow wild, nor does it volunteer well in cultivation, and it does not compete well with other plants in abandoned fields or feral environments, seldom persisting more than a few growing seasons (Pujol et al., 2002; Andersson and de Vicente, 2010). Low seed production and seed dormancy limit the ability of cassava to spread to unmanaged ecosystems and persist there (Chavarriaga-Aguirre and Halsey, 2005). Cassava appears to possess only one of Baker's characteristics of weeds, namely discontinuous germination and long-lived seeds, and cassava is not considered to be a weedy species, neither in an agricultural setting nor in the wild (Halsey et al., 2008). Originally, *M esculenta* ssp. *flabellifolia* had been proposed as an escapee from cultivated cassava (Nassar, 2002), but various taxonomic and biosystematic studies seem to agree that *flabellifolia* is most likely the wild progenitor of cassava (Roa et al., 1997; Olsen and Schaal, 1999; Allem, 2002; Andersson and de Vicente, 2010).

Some species (e.g. *M. pohlii*, *M. zehntneri* and *M. grahamii*) can be invasive in newly disturbed areas (Nassar and Ortiz, 2006; Andersson and de Vicente, 2010), while others are known to survive drought and fire (Janick and Byrne, 1984).

Agronomic, silvicultural and other intensively managed ecosystems where the species is grown or occurs, and management practices

Cultivation and management practices

Generally, cassava requires high levels of sunlight and high temperatures, adequate soil fertility and rainfall during crop establishment to produce acceptable agronomic yields (Fermont et al., 2009). Typically, the crop is grown with little or no supplemental irrigation, pesticides or fertilizers (Howeler, 2002, 1991; Leihner, 2002), but inputs such as fertilizer and water, the use of improved varieties and weed management can significantly improve yields (Fermont et al., 2009). The use of cropping methods such as mulching, intercropping, conservation tillage and contour planting may improve production under certain circumstances, but the use of these methods varies by locality, and little research has been done to optimise such practices (Janick and Byrne, 1984; Howeler, 2002).

It is common for cassava to be intercropped with other annual crops such as maize, rice, sorghum or pulses, or with perennial groundcovers, to minimise the soil erosion that can occur when cassava is grown alone (Leihner, 2002). However, because cassava establishes more slowly than many of the crops it is grown with, the timing of the plantings must be managed so that the developing cassava plants are not subject to excessive shading, causing the plant to divert photosynthesis into the production of shoots and leaves rather than storing it as starch in the roots (Alves, 2002; Lebot, 2009). The cultivar and its associated growth habit also affect the success of intercropping, because taller varieties and those with an erect growth habit may be less affected by the companion crop (Leihner, 2002).

Generally, a field intended for cassava production is prepared by slashing and burning or by disking and ploughing. Depending on the size of the farm and the farmer's resources, land preparation may be done by hand or with animal-drawn or mechanized equipment. Smallholder farmers may do little land preparation prior to planting, and some growers may plant the next season's crop while harvesting. However, on large-scale

farms under permanent cropping, ploughing to loosen the soil and improve drainage is more common, since cassava does not tolerate waterlogged soils (Lebot, 2009). Ploughing also increases the ease with which the crop can be harvested and therefore may be worth the extra effort for smallholder farmers, who generally harvest by hand (Lebot, 2009).

Cassava is grown on a variety of soils, and it tolerates marginal, low-fertility, acid soils better than many other staple crops (Janick and Byrne, 1984; El-Sharkawy, 2004). However, cassava is known to be sensitive to soils with high pH (greater than 7.8) and elevated conductivity and/or sodium (Janick and Byrne, 1984; Howeler, 2002). Cassava removes less nitrogen and phosphorous per tonne of dry matter (DM) produced than other common crops, and its efficient use of soil nutrients, especially phosphorous, is attributable to its association with soil mycorrhizae (Howeler, 2002; 1991). Cassava responds favourably to added fertilizer, especially potassium, but over-fertilization, especially with nitrogen, can increase leaf growth at the expense of root formation and increase root cyanide content (Howeler, 2002, 1991; Nassar and Ortiz, 2006).

Competition from weeds is recognised as a major limitation on cassava yields (Fermont et al., 2009). Herbicide use, although effective for increasing yields, is more common on larger farms, whereas on smaller farms, weeds are typically managed by manual weeding, mulching or other less-expensive but more labour-intensive methods (IITA, 2000; Leihner, 2002; Taylor et al., 2004). Disease control in cassava is generally accomplished through the use of resistant varieties, selecting planting materials from plants without disease symptoms, early removal of diseased plants and crop rotation (Leihner, 2002).

Vegetative and seed propagation

Cassava storage roots cannot be used for propagation, since the plant will not regenerate from root tissue; instead, mature, woody stems are harvested and cut into short "stakes" (15-30 cm) to be used for planting the next crop (Alves, 2002). A mature cassava plant may provide 10-20 stakes (Lebot, 2009). The stakes must be handled with care, as their quality can rapidly deteriorate due to desiccation, bruising and peeling. Whole stems that have been harvested can be stored for several months in cool, moist conditions and with chemical protection from insects and fungi, without significant loss of viability (Leihner, 2002).

Planting is done by placing stakes into the soil vertically, inclined or buried horizontally, on flat or ridged soil beds, usually at the beginning of a rainy season (Keating, Wilson and Evenson, 1988) (Figure 3.4). Depending on soil type, the planting orientation can influence the ease with which the roots may be harvested (IITA, 1990). In addition, vertical or inclined planting of the cuttings encourages plants with a single stem, while horizontal planting often results in multiple-stemmed plants (Lebot, 2009). Germination and early growth of the plants from stakes depends on endogenous nutrients stored in the stems rather than on soil nutrients, so the success of the planting is determined by the quality of the cuttings (El-Sharkawy, 2004).

The cuttings sprout in one to two weeks, and the first leaves begin to expand within 30 days. The canopy closes in three to four months, depending on the variety and the local environmental conditions. For the first month or two, the developing plants produce only fine-textured roots, but eventually a number of these roots, depending on the variety, begin secondary growth and starch accumulation. The onset of starch accumulation coincides with a decrease in the ability of storage roots to absorb water and nutrients

(Alves, 2002; El-Sharkawy, 2004). Development of storage roots begins with secondary growth of fibrous roots and starch deposition, which starts about 25-40 days after planting (DAP) in many cultivars (Cock, 1984). Storage root thickening begins when the supply of photoassimilates exceeds the requirements for shoot growth (Cock et al., 1979; Lian and Cock, 1979). Onset of storage root bulking is noticeable two to four months after planting when the new storage roots are at least 5 mm thick. Most of the translocation of carbohydrates to the storage roots occurs 180-300 DAP (Lebot, 2009).

Figure 3.4. **Cassava shoots sprouting from stakes**

Source: Courtesy ILSI Research Foundation-CERA.

Planting density can range from 5 000 to 40 000 cuttings per hectare, depending on the cultivar, the soil quality and the intended use of the crop. Lower planting densities (< 12 500 plants/ha) favour storage root production while higher planting densities (> 12 500 plants/ha) are used to maximise stake production (Keating, Wilson and Evenson, 1988; Leihner, 2002; Nassar and Ortiz, 2006; Odedina et al., 2009).

Stem cuttings are not necessarily taken from every plant in the field. In fact, only a small minority of the plants may serve as the source for the farmer's next season's crop (Elias, Panaud and Robert, 2000). In addition, it is not unusual for growers to exchange stem cuttings with their neighbours and with neighbouring communities, resulting in fields that contain mixtures of the local landraces (Andersson and de Vicente, 2010). Although farmers typically prefer high-yielding varieties, they may maintain lower yielding varieties in parallel with more productive varieties, due to cultural preferences such as taste or cooking quality. This practice of keeping several different varieties in production at the same time, often in the same field, is one way farmers manage the risk of a catastrophic crop loss (Elias, Panaud and Robert, 2000).

Botanical seed is not typically used for commercial propagation. Genetically, any particular cassava genotype is extremely heterogeneous (Kawano et al., 1978), and propagation from sexual seed results in a wide and unpredictable diversity of phenotypes. This diversity is of interest to breeders but presents difficulties for farmers (Ceballos et al., 2004). During the growing season, it is not unusual for seedling cassava plants to grow up among the vegetatively propagated plants. These seedlings may have germinated from seeds released by the crop itself or from seeds in the soil seed bank, and it is likely that these new seedlings are genetically different from their parental stock. In

addition, because many of the most problematic cassava diseases are passed from one crop to the next via vegetative propagation, such seedlings may be relatively disease-free (Elias, Panaud and Robert, 2000). Farmers may harvest stem cuttings from the seedling-derived plants displaying favourable agronomic characteristics and replant these cuttings with the next season's crop (Olsen and Schaal, 1999). In this way, farmers incorporate genetic variability from sexual reproduction into existing landraces. In regions where wild *Manihot* plants are prevalent, this practice may function to facilitate gene flow between cultivated plants and nearby wild plants (de Silva, Bandel and Martins, 2003; Duputié et al., 2007; Olsen and Schaal, 2007; Sardos et al., 2008). Alternatively, some farmers separate stem cuttings from these seedlings to be multiplied as a new variety (Elias and McKey, 2000; Elias et al., 2001).

Harvesting and post-harvest handling

Typically, a cassava crop produced in humid, lowland tropical regions can be harvested many months earlier than a crop grown in drier, cool highland areas (Alves, 2002). Depending on the cassava genotype, environment, soil type and intended use, the storage roots (Figure 3.5) may be harvested 6-36 months after planting. Farmers may leave a percentage of the plants standing, treating them as a perennial crop, and thereby storing food underground (Janick and Byrne, 1984; Nassar and Ortiz, 2006; Sardos et al., 2008). Some farmers may harvest only a few roots from a plant, covering the remaining roots with soil for future harvesting. However, with increasing age and unfavourable conditions, such as moisture stress, storage roots become lignified and less desirable for consumption, and the plants become more susceptible to lodging and rot (Lebot, 2009).

Figure 3.5. **Harvested cassava plant, showing roots**

Source: Courtesy ILSI Research Foundation-CERA.

Once harvested, cassava roots of many varieties undergo what is referred to as post-harvest physiological deterioration, or PPD (Lebot, 2009). Within 24-72 hours of harvest, polyphenol oxidase catalyses the formation of various polyphenolic compounds: pigments, quinones and tannins. These substances as well as various secondary metabolites, such as coumarin and scopoletin, which are also synthesised at this time, together render the root tissue inedible (Alves, 2002; Reilly et al., 2004). Heat treatments, anaerobic storage and treatments with polyphenol oxidase inhibitors – such as ascorbic acid, glutathione and potassium cyanide – can reduce the severity of PPD (Alves, 2002).

The incidence of PPD can be reduced by pruning the plants to a height of 200-300 cm, up to three weeks before roots are harvested (Marriott, Been and Perkins, 1979). This practice seems to increase the sugar/starch ratio in the roots and reduces losses from PPD (El-Sharkawy, 2004); however, pre-harvest pruning can negatively impact taste and cooking quality of the cassava roots (van Oirschot et al., 2000).

Centres of origin and diversity

Pinpointing cassava's origin has been complicated by inconclusive anthropological data and the difficulty in obtaining intact archaeobotanical samples from the humid lowland regions in Central and South America where cassava has been historically grown. Tissue samples are more easily obtained in arid regions, but it is thought that these areas are not the origins of domestication (Janick and Byrne, 1984; Olsen and Schaal, 1999; ITIS, 2012).

Concerning the origin of cassava, three questions need to be addressed: the botanical origin (i.e. the wild species from which cassava descended); the geographical origin (i.e. the area where the progenitor evolved in the geological past); and the agricultural origin (i.e. the area of initial cultivation/domestication of the wild ancestor by Amerindians).

Botanical origin

Originally, the entire genus was thought to have arisen via allopolyploidisation, possibly resulting from a cross between two related species (Janick and Byrne, 1984; Nassar and Ortiz, 2006). For many years, the accepted hypothesis was that cassava resulted from one or more hybridisation events of wild *Manihot* or other species (Rogers and Appan, 1973). It was proposed that the modern cultivated cassava, *M. esculenta* ssp. *esculenta*, originated directly from the extant wild subspecies *M. esculenta* ssp. *flabellifolia* (Allem, 1994), and this close relationship has since been supported by additional studies (Roa et al., 2000; 1997). The use of molecular tools such as amplified fragment length polymorphism (AFLP) to estimate genetic relationships of *M. esculenta* indicates that the cultivated species has a single ancestor, *M. esculenta* ssp. *flabellifolia* (Olsen and Schaal, 1999; Duputié et al., 2007).

Agricultural origin

The origin of domestication of cassava had been disputed for many years. However, recent evidence now points to an origin in the Amazon region of South America (Allem, 2002; Hillocks, 2002). It is currently assumed that there is only one domestication site for cassava, possibly along the southern border of the Amazon basin, where *M. esculenta* ssp. *flabellifolia* plants were originally collected from the wild, domesticated and multiplied by vegetative propagation (Olsen and Schaal, 1999; Elias, Panaud and Robert, 2000; Allem, 2002). Archaeological findings and other data indicate that the domestication of cassava started approximately 5000-7000 years BCE (Lathrap, 1970; Gibbons, 1990). A detailed molecular analysis based on the single-copy nuclear gene encoding glyceraldehyde 3-phosphate dehydrogenase (Olsen and Schaal, 1999) indicated that cassava was domesticated specifically from populations of *M. esculenta* ssp. *flabellifolia* occurring along the southern rim of the Amazon basin in the Brazilian states of Acre, Rondônia and Mato Grosso, and likely extending south into Bolivia. Later studies have confirmed a southern Amazonian domestication site (Olsen and Schaal, 2001; Léotard and McKey, 2004).

Geographical origin

Central Brazil, with its large number of wild *Manihot* species, is the likely primary centre of diversity of cassava (Nassar, 2000). There is evidence in the literature that cassava has been in cultivation in northern Amazonia for as long as 1 000 years and that migration of native peoples from this region to Central America and central Brazil, where wild *Manihot* species were already present, resulted in the creation of new centres of diversity (Nassar, 2000). These domesticated varieties were subsequently moved during migrations of native peoples, allowing hybridisation to occur between the cultivars and local wild relatives (Janick and Byrne, 1984; Nassar, 2002, 2000).

In the 16th century, the Portuguese brought domesticated varieties of cassava from Brazil to West Africa, from which it was spread across the sub-Saharan region (Hillocks, 2002; Okogbenin et al., 2007). The Spanish brought cassava from Central America in the 16th century to the Philippines, from which it spread to South East Asia, Indonesia and the Pacific Island countries (Janick and Byrne, 1984). Cassava was introduced to east Africa in the 17th century through Madagascar, Zanzibar and other Indian Ocean islands (Jennings, 1976). By the 18th century, movement via ocean routes brought cassava to mainland eastern Africa, and soon after to India, Java and South East Asia (Purseglove, 1968; Janick and Byrne, 1984).

Reproductive biology

Generation time under natural circumstances and where grown or managed

Although some cultivars of cassava can be managed as an annual crop, harvested in six months only after the stem cuttings are planted, it is actually a perennial shrub (Alves, 2002). Cassava undergoes annual cycles of vegetative growth, accompanied by carbohydrate storage in the roots, followed by a period of dormancy during cool, dry conditions (Lebot, 2009). Some growers may leave mature plants in the soil for up to 36 months, storing the roots for harvest later (Janick and Byrne, 1984; Nassar and Ortiz, 2006; Sardos et al., 2008; Lebot, 2009).

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

Flowering time for cassava varies widely with the cultivar. Some varieties flower as early as 2 months after planting, while others may flower as long as 24 months after planting. Flowering between 6 and 18 months after planting is typical for the species (Janick and Byrne, 1984). Once flowering is initiated, an individual plant may produce flowers for over two months (Alves, 2002).

Generally, grower selection of cuttings for vegetative propagation has resulted in plants with reduced branching. Since inflorescences form at branch points in the stem, long-term vegetative propagation selects against flower formation and the ability of individual plants to reproduce sexually (Duputié et al., 2007; Olsen and Schaal, 2007; Halsey et al., 2008). In branching varieties, branching begins as early as two months after planting, and flower formation occurs approximately one week later, at the branching points (Halsey et al., 2008). However, early inflorescences are known to abort, so that functional flowers are generally seen emerging from secondary branch points (Lebot, 2009).

Floral biology

Cassava is monoecious, bearing separate female and male flowers on the same plant (Figure 3.6). The flowers are borne together in the inflorescences, with the pistillate flowers beneath the staminate flowers. A flower bud typically forms where the plant branches, so that more highly branched genotypes flower more prolifically than those with sparsely branched habit. The onset of branching, and therefore flowering, is prompted by long days (up to 16-hour day length) in some cultivars (Alves, 2002). The number of flowers produced by a plant varies among varieties, and some genotypes have never been observed flowering (Kawano, 1980; Alves, 2002). Flowering may also be influenced by environmental factors, so that a particular clone may not flower at all in one environment, produce aborted flowers under other conditions, or produce numerous flowers and set seed in another environment (Halsey et al., 2008). It appears that moderate temperature (approximately 24°C) is most suitable for flowering (Alves, 2002).

Female flowers have five tepals, which can be red, yellow or purple, and a sticky stigma which secretes nectar on the day the flower opens, attracting insect pollinators (Lebot, 2009). The pistillate flowers are approximately 13 x 8 mm in size (Janick and Byrne, 1984). The male flowers are half the size of the female flowers, approximately 5 mm, but are more numerous and each flower has ten stamens, borne in two rings (Janick and Byrne, 1984; Alves, 2002).

Figure 3.6. **Cassava female and male flowers**

A. Female flower B. Male flower

Source: © Ton Rulkens.

Pollination, pollen dispersal, pollen viability

The female flowers open for approximately one day, and the stigma is receptive throughout that time. Fertilization occurs 8-19 hours after pollination (Andersson and de Vicente, 2010).

Individual cassava inflorescences display protogyny, with female flowers opening one to two weeks before the staminate flowers on the same inflorescence. However, because a single plant usually has more than one inflorescence, male and female flowers on the same plant may open at the same time (Alves, 2002). Therefore, while cassava is generally thought to be an outcrossing species, natural self-pollination may also occur, depending on the cultivar (Janick and Byrne, 1984).

The pollen grains are large, ranging from 90-148 µm in size (Hahn, Bai and Asiedu, 1990; Alves, 2002; Halsey et al., 2008; Vieira et al., 2012a). Typically, pollen viability is lost quickly after shedding; for example, Leyton reported 97% seed set with newly collected pollen, 56% seed set with pollen stored for 24 hours at 25°C, and only 0.9% seed set after 48 hours of storage (Leyton, 1993; Nassar and Ortiz, 2006). As a result, cassava breeders typically use pollen for crosses within one hour after collection (Halsey et al., 2008; Andersson and de Vicente, 2010).

Vieira et al. (2012a) conducted a study on viability, production and morphology of the pollen grains of five varieties of cassava and accessions of six *Manihot* wild species and subspecies (*M. anomala*, *M. dichotoma*, *M. esculenta* ssp. *flabellifolia*, *M. esculenta* ssp. *peruviana*, *M. tomentosa* and *M. violacea*). In general, the wild accessions produced more (579-3 638 grains per flower) and larger (132-163 µm) pollen grains compared with the cassava varieties (613-1 193 grains and 129-146 µm). The number of pollen grains for the cultivated cassava varieties was similar to *M. esculenta* ssp. *flabellifolia* and *M. esculenta* ssp. *peruviana*, but significantly smaller than the wild accessions of *M. dichotoma*, *M. tomentosa* and *M. violacea*. The lower pollen production in the cultivated cassava varieties, *M. esculenta* ssp. *flabellifolia* and *M. esculenta* ssp. *Peruviana*, could represent one of the consequences from the initial steps in the domestication process. The process favours the vegetative propagation of the species to the detriment of sexual propagation and, consequently, the production of pollen.

The pollen grains of cassava are sticky, which limits wind pollination (Halsey et al., 2008). Various species of bees and wasps appear to be the main pollinators of cassava in both Africa and Latin America, including *Apis mellifera*, *Polybia* spp. and *Polistes* spp. (Janick and Byrne, 1984; Nassar and Carvalho, 1990; Halsey et al., 2008; Andersson and de Vicente, 2010). Most pollen foraging occurs over a distance of 1-5 metres (Andersson and de Vicente, 2010), and when cassava plants were grown spaced at 2 x 2 m, both insect-mediated outcrossing as well as a smaller amount of self-pollination are observed (de Silva, Bandel and Martins, 2003).

Reproductive isolation of cassava can be effectively accomplished by a number of means, including isolation distance, destruction of plants prior to flowering, removal of flower buds and bagging of flowers. Kawano et al. (1978) conducted detailed research on reproductive isolation distances in cassava using a very large germplasm collection as a pollen source to eliminate biases related to flower opening, potential genetic incompatibilities and limited pollen pool. They observed measurable gene flow at 1 m, but found no gene flow at 30 m and 500 m, suggesting that an isolation distance of 30 m is adequate to ensure genetic isolation of cassava in field experiments. Other data indicate that reproductive isolation of wild *Manihot* (the pollen source) and feral cassava could be accomplished using a distance of 60 m (Duputié et al., 2007). Because wild *Manihot* species begin flowering earlier and flower more profusely than cassava, measurements of gene flow from wild *Manihot* to cassava would likely overestimate actual gene flow that may occur in cassava to cassava situations (Fregene, 2010).

Seed production, and natural dispersal of fruits, cones and seeds

The fruit of cassava (Figure 3.7) is a tricarpellary capsule, and each locule contains one ovule; however, it is common for capsules to contain fewer than three seeds (Kawano, 1980). The fruit reaches maturity two to three months after pollination, and the

fruit dehisces explosively, although seed typically falls to the ground near the mother plant (Alves, 2002).

Figure 3.7. **Fruit of cassava**

Source: © Ton Rulkens.

Ants are attracted to the seeds, which bear an edible oil body called the caruncle. The ants assist in seed dispersal by bringing the seeds to their nests, resulting in seed movement up to several meters; however, ants' contribution to cassava seed dispersal appears to vary by species and distance to nest entrances (Elias and McKey, 2000; Elias et al., 2001). Ant dispersal is associated with fire-adapted species, since the movement of seeds into ants' underground burrows protects the seeds from high temperatures occurring during bush fires (Pujol et al., 2002).

Some birds, specifically doves, may also have a role in the dispersion of the seeds (Elias and McKey, 2000; Andersson and de Vicente, 2010).

Seed viability, longevity and dormancy; natural seed banks; germination; seedling viability and establishment

Cassava seed is subject to a dormancy period of various lengths, depending on the genotype. Seeds falling to the soil become dormant, forming seed banks from which plants may germinate (Pujol et al., 2002; Andersson and de Vicente, 2010). Seeds can remain viable when stored under ambient conditions for up to one year, although germination percentages may decline substantially after six months (Kawano, 1980; Rajendran, 2000). Seeds will remain viable and dormant for several years under cool (4°C), humid (70-80% relative humidity) and dark conditions, which are unfavourable for germination (Janick and Byrne, 1984; Pujol et al., 2002; Halsey et al., 2008; Lebot, 2009).

Seed scarification has mixed success in breaking dormancy, but several successful thermal treatments, involving exposure to 35°C, have been developed to shorten dormancy and increase germination frequency (Pujol et al., 2002; Nassar and Ortiz, 2006). The fact that germination is stimulated by dry heat suggests that cassava has evolved where fire cycles were common (Pujol et al., 2002).

Asexual propagation (apomixis, vegetative reproduction)

Because of the propensity for natural inter-varietal and interspecific hybridisation, cassava varieties are preserved through vegetative propagation. Farmers generally do not establish cassava crops using seed (Janick and Byrne, 1984; Halsey et al., 2008). As previously stated, many cassava varieties have become adapted to vegetative reproduction and flower little, if at all (Lebot, 2009).

Apomixis occurs frequently in *Manihot* species, including *M. esculenta*, and data indicate that the mechanism is apospory, the development of the gametophyte from the sporophyte without meiosis (Nassar, 2000). Apomixis is thought to have contributed to the rapid speciation of the genus by enabling interspecific *Manihot* hybrids living in naturally occurring micro-environments to develop into new species (Nassar, 2002).

Genetics

All *Manihot* species have the same chromosome number (2n = 36), and the species generally display normal diploid meiosis (Rogers and Fleming, 1973; de Carvalho and Guerra, 2002). Although *M. esculenta* has also been described as an allotetraploid with basic chromosome number 1n = 9 (Umanah and Hartmann, 1973), studies conducted on the meiotic behaviour of several cassava genotypes observed the formation of 18 bivalent chromosomes typical of a diploid. The amount of hybridisation noted between cassava and its wild relatives suggest that interspecific hybridisation barriers are fairly weak. In fact, no incompatibility systems have been identified in *Manihot* that prevent or inhibit crossing between species, and cassava chromosomes are observed to pair with those of even distant relatives (Janick and Byrne, 1984). Natural and artificial hybrids of cassava and *M. glaziovii* have been recorded (Lefèvre and Charrier, 1993; Second et al., 1997), and additional discussion of intraspecific crosses within the genus is presented in the next section.

Cassava does occasionally exhibit meiotic irregularities, possibly due to the almost exclusive use of vegetative propagation to produce the crop, which can result in the accumulation of somatic mutations (Hahn, Bai and Asiedu, 1990; Olsen and Schaal, 2007; Sardos et al., 2008). As a result, many cultivars display some sterility, typically due to one of several mechanisms by which the male flowers fail to mature and produce viable pollen (Janick and Byrne, 1984; Olsen and Schaal, 2007; Lebot, 2009).

The genetics of cassava and its relatedness to wild *Manihot* species has been examined using a variety of molecular tools, including isozyme analysis (Olsen and Schaal, 1999; Cabral et al., 2002); RAPD (random amplified polymorphic DNA) (Nassar, 2000); RFLP (restriction fragment length polymorphism) (Beeching et al., 1993; Fregene et al., 1997); AFLP (amplified fragment length polymorphism) (Roa et al., 1997; Elias, Panaud and Robert, 2000); and SSR (simple sequence repeat) and microsatellite markers (Elias et al., 2001; Duputié et al., 2007; Otti et al., 2011). Marker-assisted cassava breeding can assist with the selection of appropriate parents and ultimately in the production of improved varieties (Lebot, 2009). Approximately 96% of the protein-coding sequences of one variety of cassava have been sequenced, revealing over 30 000 predicted genes (Prochnik et al., 2012). There are currently no studies available that show evidence of organellar inheritance of agronomically important traits in cassava.

Because of the propensity for natural interspecific hybridisation, cassava is highly heterozygous (Janick and Byrne, 1984; Alves, 2002). Many traditional varieties, when tested using microsatellite markers, have been found to be polyclonal (Sardos et al.,

2008). Outcrossing within and between fields is common, and although cassava is vegetatively propagated using stem cuttings, seeds produced during the growing season or in previous seasons may fall to the ground and germinate. Because of the extent of the cassava seed bank in areas where the crop has been in cultivation for many years, some of these seedlings may represent varieties that are no longer grown (Elias et al., 2001). Thus, even with vegetative propagation, cassava fields may contain significant genetic diversity (Andersson and de Vicente, 2010).

Another source of variability comes from the difficulty in distinguishing different cassava varieties, and even different species of *Manihot*, solely by the use of morphological characteristics (Elias et al., 2001). Although some varieties have local names, the names are not indicative of genetic background, as names may be assigned to multiple varieties, or the same variety may bear several different names depending on the region where it is grown (Elias, Panaud and Robert, 2000; Sardos et al., 2008). Even the concept of a "variety" may vary from one culture to another, further complicating the understanding of cassava genetics (Peroni, Kageyama and Begossi, 2007).

Hybridisation and introgression

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

The ancestry of cassava and its relatedness to other members of the *Manihot* genus remains a topic of active research, and additional light will be shed on these questions as more sophisticated genetic tools are employed (Allem, 2002). A relatively high rate of hybridisation, combined with the naturally occurring micro-environments in South and Central America, has contributed to rapid speciation (Nassar, 2000). Apparent hybrids between cassava and its wild relatives, such as *M. zehntneri*, have been observed growing at the margins of cultivated cassava fields. Pollen movement from cassava to wild relatives and vice versa have been proposed as mechanisms by which both cultivated varieties and wild species can obtain new genetic diversity (Nassar and Ortiz, 2006).

Introgression may result in genetic enhancement of local landraces via gene flow from wild *Manihot* species; however, evidence indicates that the genetic diversity of cassava is contained within the diversity of *Manihot*, so it appears that gene introgression from wild populations into cassava is not the primary driving force for the crop's evolution globally (Olsen and Schaal, 2007). Although field observations indicate that hybrids grow larger and more vigorously than the parents, heterosis may be limited to vegetative characteristics and may not be expressed as increased fertility or reproductive fitness (Duputié et al., 2007). It is possible, however, that such hybrids may exploit new ecological niches better than the parents, eventually resulting in speciation (Nassar and Ortiz, 2006; Duputié et al., 2007).

Although manual interspecific crosses have been documented by many researchers, there is little available information regarding natural hybrids between cassava and its wild relatives (Nassar, 2003; Duputié et al., 2007). The absence of synchronous flowering has been proposed as one reason why hybridisation between cassava and its wild relatives is not seen more frequently (Nassar, 2003; Andersson and de Vicente, 2010). Many varieties of cassava have extended flowering periods, which could overlap with those of nearby wild plants, and it is proposed that greater evidence of hybridisation will be found with the increased use of molecular genetics tools (Duputié et al., 2007). Data on the viability of the seeds from presumed hybrids are generally lacking (Andersson and de Vicente, 2010), but fertile hybrids between cassava and its presumed progenitor,

M. esculenta ssp. *Flabellifolia*, have been found in nature (Duputié et al., 2007). *M. glaziovii* (*M. carthagenensis* ssp. *glaziovii*) is the only species within *Manihot* that is known to have naturalised in Africa, and natural hybrids between cassava and *M. glaziovii* have been found in Africa, although pollination frequencies are low (Halsey et al., 2008; Lebot, 2009; Andersson and de Vicente, 2010).

Experimental crosses

No genetic barriers to crosses between cassava genotypes have been identified, but manual crosses can be difficult to make due to the need for synchronous flowering (Halsey et al., 2008). In addition, the high heterogeneity of cassava can complicate breeding efforts due to uncertainty about the precise pedigree of the parental lines (Okogbenin et al., 2007). To address this heterozygosity, various molecular techniques, such as the use of microsatellite markers, are employed to verify genotypes of parental plants (Otti et al., 2011).

Some data indicate that the use of insect vectors for pollination rather than pollination by hand results in a greater number of successful hybridisations (Nassar, 2000). Bridge species, such as *Manihot neusana* which more readily cross both with *M. esculenta* and other wild *Manihot* species, can be used to move genes between species which do not cross well (Nassar, 2003). Another technique that has been observed to increase the success of manual crosses is the use of "mentor" pollen – pollen of the same species as the maternal plant that is devitalised by freezing and mixed with the pollen from the desired male parent (Nassar, 2003).

Experimental interspecific crosses between cassava and its wild relatives have been documented in the literature. Very often, considerable effort such as embryo rescue is needed to ensure success of the interspecific crosses. These crosses result in varying levels of hybrid fertility (Nassar, 2000). Spontaneous tetraploids and triploids have also been observed in the progeny of crosses between cassava and the related species *M. epruinosa* and *M. glaziovii* (Hahn, Bai and Asiedu, 1990). Some triploids show desirable qualities, such as increased vigour, higher starch accumulation and/or longer lasting leaves, and some farmers select such triploids for vegetative propagation (Lebot, 2009).

Interspecific crosses have been used in a few cassava improvement programmes. For example, genes conferring resistance to cassava mosaic disease (CMD) and cassava bacterial blight (CBB) have been moved from *M. glaziovii* into cassava (Hahn, Bai and Asiedu, 1990), and backcross derivatives from interspecific hybrids between cassava and *M. glaziovii* have been released as successful varieties in Africa, for instance TMS 30572 ("Migyera") (Jennings and Iglesias, 2002). Hybrids between cassava and *M. oligantha* show increased protein levels and reduced cyanide production in the roots (Lebot, 2009). An interspecific hybrid between cassava and *M. walkerae* was identified with delayed onset of post-harvest physiological deterioration, and several other *Manihot* species have been identified with high protein, high DM content and green mite resistance (Fregene et al., 2006).

Three accessions of *M. esculenta* ssp. *flabellifolia* were hand-crossed with 7 varieties of cassava, and the paternity of the interspecific hybrids was investigated using 24 microsatellite markers (SSRs). The rate of hybridisation success varied from 17% to 92% and the data demonstrated that SSR markers can be routinely used in breeding programmes to verify the paternity of interspecific crosses of cassava (Vieira et al., 2012b).

Information and data on introgression

There are limited studies on introgression in cassava. For natural hybridisation to take place between a wild relative and cassava, they must be in close proximity, i.e. less than 30 m (Andersson and de Vicente, 2010), and they must also be flowering simultaneously, with the concurrent presence of effective insect pollinators. When cassava was inter-planted with either *M. anomala* or *M. neusana*, putative hybrid seed was produced but seedling viability was poor, and the few surviving hybrids were identified by morphological characteristics, not by molecular methods (Nassar, 2003). Recent data have shown that through controlled hybridisations, genes for high DM content, high protein content of storage roots and delayed post-harvest physiological deterioration were introduced from wild relatives to cultivated cassava varieties (Ojulong et al., 2008; Morante et al., 2010; Okogbenin et al., 2012). In such cases, the interspecific hybrids were semi-fertile and recovered through embryo rescue techniques (Akinbo, Labuschagne and Fregene, 2010).

Plant developmental stages

Cassava is a perennial shrub that can grow indefinitely, alternating periods of vegetative growth, storage of carbohydrates in the roots and periods of dormancy. During its growth, there are distinct developmental phases. The occurrence, duration and existence of each phase depend on several factors related to varietal differences, environmental conditions and cultural practices. The plant developmental stages under favourable conditions in the field, expressed in days after planting (DAP), are as follows:

- Sprouting from stem cuttings, 5-15 DAP

  From 5-15 DAP, the first adventitious roots arise from the basal cut surface of the stake and occasionally from the buds under the soil. The first sprouting occurs 10-12 DAP, followed by the emergence of small leaves within 15 DAP (da Conceiçao, 1979).

- Beginning of leaf development and formation of root system, 15-30 DAP

  The true leaves start to expand around 30 DAP when the photosynthesis process starts to contribute positively to plant growth. Before 30 DAP, shoot and root growth depends on the reserves of the stem cutting. The fibrous roots start to grow, replacing the first adventitious roots. These new roots penetrate the soil, reaching 40-50 cm deep, and function in water and nutrient absorption (da Conceiçao, 1979). A few fibrous roots (3-14) will become storage roots, which can be distinguished from fibrous roots, beginning from 60-90 DAP (Cock et al., 1979). At 75 DAP, the storage roots represent 10-15% of total DM.

- Development of stems and leaves (canopy development), 90-180 DAP

  Maximum growth rates of leaves and stems are achieved in this period, and the branching habit and plant architecture are defined. From 120-150 DAP the leaf canopy closes (Veltkamp, 1985). Maximum canopy size and maximum DM partitioning to leaves and stems are accomplished (Távora et al., 1995). The storage roots continue to bulk. The most vegetative growth occurs during this period (Ramanujam, 1985).

- High carbohydrate translocation to roots, 180-300 DAP

Photoassimilate partitioning from leaves to roots increases, accelerating the bulking of storage roots. The highest rates of DM accumulation in storage roots occur within this period (Peressin et al., 1998). Leaf senescence increases, hastening rate of leaf fall, and in this stage the stem becomes lignified (da Conceiçao, 1979).

- Dormancy, 300-360 DAP

  Rate of leaf production is decreased in this stage. Almost all the leaves fall and shoot vegetative growth is finished. At this stage, maximum DM partition to the root is attained. This phase occurs primarily in the regions with a distinct cool, dry season (Lebot, 2009).

General interactions with other organisms

Because canopy closure in cassava fields can occur fairly late in the growing season, there is a window of time, as long as four months, during which weeds can establish, competing with the developing crop for water and nutrients. Vigorous, fast-growing cassava varieties are less sensitive to competition from weeds, but they tend to produce greater amounts of above-ground mass at the expense of storage root mass (Leihner, 2002).

Significant root yield losses can be caused by predation by mites, thrips, scales, whiteflies and mealybugs. Major diseases include cassava mosaic disease, cassava brown streak disease, cassava root rot diseases, cassava bacterial blight, anthracnose and super-elongation. Common pests and pathogens are presented in Annex 3.A1. Cassava breeders have identified resistance genes to some of the more significant insect pests and diseases of cassava in several wild *Manihot* species (Janick and Byrne, 1984). Cassava breeding and improvements obtained through biotechnological techniques or contemplated for future developments, are presented in Annex 3.A2.

Annex 3.A1. Common pests and pathogens

Because cassava is a low-value crop, it is typically grown with minimal inputs, and insecticides and fungicides are seldom used by smallholder farmers (Bellotti, 2002). In addition, yield reductions due to insects and diseases may be overshadowed by those caused by low soil fertility and moisture stress (Hillocks and Wydra, 2002). To date, smallholder farmers have relied on cultural practices and native resistance in cassava to mitigate insect pests and pathogens (Janick and Byrne, 1984), but crop losses from pests and diseases are often significant (Bellotti, 2002). Due to the genetic heterogeneity of cassava, resistance to major pests and pathogens varies widely among the hundreds of varieties in common use across the tropics. Unfortunately, even when resistant varieties are identified, farmers may be unwilling to switch varieties, mainly because the new varieties do not have other traits they prefer. The situation is worsened by the almost universal practice of vegetative propagation for cassava, which results in the accumulation of systemic infections in the crop (Bellotti, 2002; Okogbenin et al., 2007). As cassava production shifts to large-scale farms, disease and insect pressure are expected to increase.

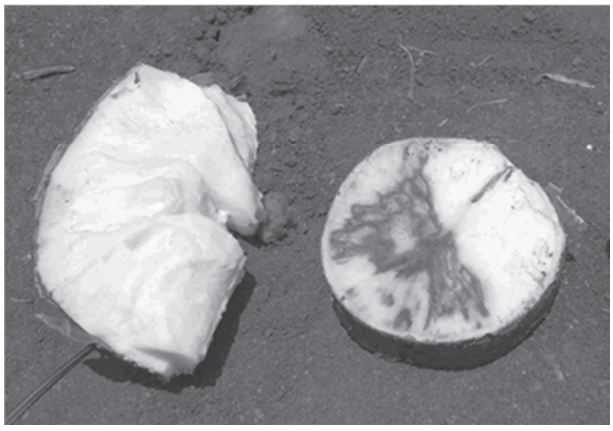
Diseases

Cassava mosaic disease (CMD) is the most significant cassava disease in Africa. It is caused by geminiviruses that are vectored by the whitefly, *Bemisia tabaci* (Taylor et al., 2004). Several cassava geminivirus species, distinguishable by serological and molecular tools, and genome sequence information, affect cassava in Africa, India and Sri Lanka. The prevailing view is that CMD is endemic to Africa and did not co-evolve with cassava in Latin America (Calvert and Thresh, 2002). The viruses causing CMD distort the leaves and restrict growth, thereby reducing root yields, but quantifying losses is difficult (Calvert and Thresh, 2002). Overall, storage root yield losses across sub-Saharan Africa were estimated at 15-24% annually, which is equivalent to 12-23 million tonnes, or an annual loss of USD 1.2-2.3 billion (Calvert and Thresh, 2002). There are several resistant varieties available, but some farmers choose to grow traditional varieties instead, in spite of their susceptibility to the disease. Cultural practices such as using virus-free planting material and culling diseased plants can help manage losses from CMD (Calvert and Thresh, 2002). Central and South American varieties are susceptible to CMD but the vector for the viruses is largely absent from the region, although *B. tabaci* and a new biotype, *B. argentifolia*, have been found in the Americas, making the need for CMD-resistant varieties even more crucial (Bellotti, 2002; Okogbenin et al., 2007). The use of resistant varieties is the most effective measure for the control of CMD in many African countries (Mahungu, Dixon and Kumbira, 1994). Two major sources of resistance genes have been used; genes derived from *Manihot glaziovii* and the CMD2 gene from West African landraces (Fregene et al., 1997; Akano et al., 2002), and some success has been achieved in moving these genes into cassava to produce highly resistant varieties (Calvert and Thresh, 2002). However, CMD often results in significant storage root yield losses that can occur even in resistant genotypes that show only mild or no foliar symptoms (Seif, 1982).

Cassava bacterial blight disease (CBB), caused by *Xanthomonas axonopodis* pv. *manihotis* (*X. campestris* pv. *manihotis*), kills both leaves and young shoots via systemic infection (Hillocks and Wydra, 2002). The disease can not only cause the loss of the root crop, but also make the stems unusable for propagation. Although less common in Asia, CBB is present in most areas where cassava is grown. The bacterium is vectored by grasshoppers and can also be spread via contaminated stakes and seed (Hillocks and Wydra, 2002; Lebot, 2009). CBB can be managed via crop sanitation, cultural practices and crop rotation, and seeds can be effectively disinfected using heat treatments (Hillocks and Wydra, 2002). Resistant varieties have been identified, but resistance has been overcome by increasingly virulent strains of the bacterium (Fregene and Puonti-Kaerlas, 2002; Hillocks and Wydra, 2002).

Cassava brown streak disease (CBSD) is a viral disease that causes elongated necrotic lesions in storage roots (Figure 3.A1.1), and variable symptoms on stems and leaves. CBSD is caused by two virus species: cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV), classified in the genus *Ipomovirus* (Family *Potyviridae*) (Mbanzibwa et al., 2009; Monger et al., 2010; Winter et al., 2010). Yields are reduced by severe infections (Hillocks et al., 2008), but the more important impact is on the quality of storage roots, since the necrotic lesions render roots unusable (Calvert and Thresh, 2002). The disease is spread through the planting of infected stem cuttings (Taylor et al., 2004), and there is evidence of spread via an arthropod vector, possibly whiteflies (Calvert and Thresh, 2002). Although known to have been present in east Africa since the 1930s, CBSD was mostly confined to the coastal regions and around Lake Malawi (Legg et al., 2011) until a new outbreak was identified in Uganda in 2004 (Alicai et al., 2007). Since that time, the disease has developed to epidemic proportions, representing a significant constraint to cassava production throughout Eastern and Central Africa (Lebot, 2009; Campo, Hyman and Bellotti., 2011; Legg et al., 2011; Jarvis et al., 2012).

Figure 3.A1.1. **Healthy cassava root tissue (left) and brown streak disease infected (right)**



Source: Courtesy ASARECA.

Three other viral diseases, cassava common mosaic disease, cassava vein mosaic disease and cassava frogskin disease, are of lesser importance in terms of crop loss. They occur in South America and are generally controlled by planting virus-free stock and culling infected plants. There is no effective resistance to any of these diseases (Calvert and Thresh, 2002; Lebot, 2009).

Angular leaf spot, caused by *X. campestris* pv. *cassava*, is prevalent in Africa and can cause defoliation when severe (Lebot, 2009). However, unlike cassava bacterial blight, angular leaf spot does not result in systemic infection of the plant (Hillocks and Wydra, 2002).

Stem and root rots, caused by *Erwinia carotovora* ssp. *carotovora*, occur in South America and Africa and result in yield losses and the destruction of planting stock. The bacterium is vectored by fruit flies (e.g., *Anastrepha* spp.), and the planting of fruit fly resistant varieties and spraying to kill the flies can help control the disease (Hillocks and Wydra, 2002; Lebot, 2009).

Various leaf spot and stem diseases of cassava, occurring worldwide, are caused by several species of fungi such as *Cercospora*, *Phoma* and *Colletotrichum*. Disease severity is generally worse in humid regions, but infestations resulting in significant yield loss are uncommon. Some resistant varieties have been identified, but there are no other effective control measures (Hillocks and Wydra, 2002; Lebot, 2009).

Several fungal root rot diseases are caused by *Phytophthora*, *Pythium*, *Fomes*, *Sclerotium* and *Armillariella*, and when severe, these pathogens can cause significant or complete loss of storage roots. However, these diseases occur sporadically and usually under specific conditions, such as in poorly drained soils or in recently cleared forest land. Varieties differ in resistance to these diseases (Hillocks and Wydra, 2002).

Arthropods

Many arthropod pests have co-evolved with cassava, and these species are much more prevalent in South America than in either Asia or Africa. However cassava is also subject to predation by generalist feeders wherever it is grown. Generally, insect damage is more severe in drier climates and during dry seasons in humid climates, and plants may be able to recover from predation with adequate rainfall or irrigation (Bellotti, 2002).

Managing insect damage in cassava is extremely challenging, especially for smallholder farmers. Pesticide use is usually precluded by the high cost; moreover, pesticides may disrupt the activity of existing natural enemies in the environment. For specific pests, cultural practices such as intercropping may mitigate crop losses, but these practices are not universally effective, and large-scale production may preclude the use of some of these practices (Bellotti, 2002). Resistant cultivars are not available for most arthropod pests and while some resistance has been found in wild *Manihot* species, moving these traits into desirable cassava varieties is a long process (Bellotti, 2002).

The cassava green mite is the common name for approximately 40 different mite species, for example *Mononychellus tanajoa* (cassava green mite) present in South America and Africa, and *Tetranychus urticae* present in South America and Asia. Mites, which harm the growing points and young leaves, can cause stunting when infestations are severe (Bellotti, 2002). They can be controlled by predatory mites (*Typhlodromalus aripo* and *T. manihoti*). Control by a fungus (*Neozygites tanajoae*) is the subject of ongoing research (Bellotti, 2002; Nassar and Ortiz, 2006; Lebot, 2009), and a few varieties have been identified with low to moderate mite resistance (Bellotti, 2002).

Phenacoccus manihoti and *P. herreni* are the predominant species of mealybug in South America and Africa, causing leaf damage, shoot malformation and even yield losses when infestations are severe. There is little native resistance to mealy bugs

(Bellotti, 2002), but various parasitic wasps (e.g. *Apoanagyrus lopezi*) have been effective in their control (Lebot, 2009).

Many species of whiteflies, of which *Aleurotrachelus socialis* is predominant, cause significant cassava crop losses due to photosynthate loss from phloem feeding. Research is underway to identify varieties with useful resistance as well as appropriate whitefly parasitoids (Bellotti, 2002). The whitefly, *B. tabaci*, is a major pest of cassava, particularly in eastern Africa, where it is responsible both for the transmission of viruses that cause CMD and CBSD, and increasingly for direct damage due to feeding by high populations (Omongo et al., 2012).

In South America, the cassava stem borer (*Chilomina clarkei*) causes damage by feeding internally on stems, resulting in stem breakage. Although borer damage does not usually result in significant yield loss, they do reduce the amount and quality of planting material available for the next year's crop (Bellotti, 2002). Traditional pesticide sprays are ineffective against the borer because the insect causes much of its damage while inside the stem, protected from externally applied sprays (Taylor et al., 2004; Lebot, 2009). Research is ongoing to identify effective natural enemies and resistant varieties (Bellotti, 2002).

The hornworm (*Erinnyis ello*) is a serious cassava pest in South America, which feeds on young leaves and stems and can completely defoliate the plant. Although the plants typically recover, the weakening of the plant can result in large yield losses. Effective and inexpensive control of hornworm has been achieved using sprayed suspensions of a Baculovirus (Bellotti, 2002; Lebot, 2009). Control by natural predators has limited effectiveness, due to the migratory behaviour of the hornworm adults, resulting in the deposition of large numbers of eggs that hatch while predator populations are too low to provide control. Better monitoring of hornworm migrations and synchronizing predator release with egg laying may result in more effective control (Bellotti, 2002).

Cyrtomenus bergi is polyphagous, feeding on storage organs of many crops. In cassava-growing areas, it is known as the cassava burrower bug. Cassava is not its preferred host, and the bug tends to avoid cassava varieties that produce higher levels of cyanogenic glucosides. Root feeding allows infection by any of several soil-borne fungal pathogens, causing lesions in the root tissue and reducing starch content. Severe infestations by the burrowing bug can cause significant crop losses (Bellotti, 2002).

Other

Yield losses due to nematodes such as *Meloidogyne* and *Pratylenchus* are difficult to measure, and nematodes are not generally regarded as serious pests on cassava. However, crop damage can increase over many seasons, as nematode populations build up, and when this occurs, the planting of resistant varieties is advisable (Hillocks and Wydra, 2002).

Annex 3.A2. Biotechnological developments

Given the importance of cassava as a source of dietary calories in the tropics, there is a great deal of interest in using biotechnology to improve the crop to increase nutritional quality, reduce pre- and post-harvest losses, decrease cyanogenic potential of the edible parts of the plant, and to develop disease-resistant varieties (Taylor et al., 2012, 2004; Lebot, 2009).

Although cassava was at first recalcitrant to plant tissue culture methods, using plant transformation to obtain transgenic cassava plants became possible in the mid-1990s. Typically, researchers use embryogenic tissue from a variety of explants, and cell transformation is accomplished using biolistic methods or *Agrobacterium tumefaciens* (Fregene and Puonti-Kaerlas, 2002; Taylor et al., 2004). Challenges to the use of biotechnology to produce improved cassava varieties include the requirement that gene expression remain at effective levels after many generations of vegetative reproduction, and the difficulty in achieving homozygosity in a largely heterozygous crop. The development of double haploid cassava lines is under investigation to assist with this limitation (Taylor et al., 2004; Aerni, 2006). Also, transgenic traits must be made available in a wide range of varieties that farmers want to use. Ideally, important landraces would be transformed with traits of agronomic significance, but there can be considerable variability in the culturability of individual landraces, even when the landraces are related (Taylor et al., 2004).

Nutritional improvements

There is ongoing research into the enhancement of micronutrient and vitamin content (such as zinc, iron and vitamin A/ß-carotene) of cassava through genetic engineering (Fregene and Puonti-Kaerlas, 2002; Taylor et al., 2004; Sayre et al., 2011). Modifying starch quality and enhancing the production of sugars in the storage roots is also under investigation (Fregene and Puonti-Kaerlas, 2002).

To increase protein content of cassava storage roots, tissue-specific production of an artificial storage protein is being attempted (Fregene and Puonti-Kaerlas, 2002; Taylor et al., 2004; Sayre et al., 2011). Efforts are underway to increase starch synthesis and accumulation, for both food and industrial purposes, and to reduce starch grain size, largely for industrial uses.

In addition to directly improving the storage root quality, there are efforts underway to improve foliage quality, specifically the longevity of the leaves. Leaves that remain photosynthetic longer contribute to higher root yields, and in regions where the leaves are also consumed, long-lived leaves add to the overall value of the crop (Fregene and Puonti-Kaerlas, 2002).

Efforts to reduce the release of cyanide from cassava tissues focus on either reducing the production of the cyanogenic glycosides or increasing the rate of breakdown of the glycosides. In the first instance, the approach is to use anti-sense constructs to reduce the synthesis of a cytochrome P450 that catalyses the first step in the synthesis of linamarin and lotaustralin. In the second case, the approach is to increase the synthesis of

hydroxynitrile lyase, which catalyses the breakdown of acetone cyanohydrin into acetone and hydrogen cyanide (Fregene and Puonti-Kaerlas, 2002; Taylor et al., 2004).

Pre- and post-harvest losses

Lepidopteran insects, particularly stem borer (*Chilomina clarkei*) and hornworm (*Erinnyis ello*), cause major cassava crop losses in Latin America. Lepidopteran insect control using a transgene from *Bacillus thuringiensis* producing one of the Bt proteins is under investigation, and experimental plants display resistance to both species (Fregene and Puonti-Kaerlas, 2002; Taylor et al., 2004).

Cassava mosaic disease and cassava brown streak disease are the greatest constraints to cassava production, and resistance to both diseases is being addressed from a variety of gene-silencing approaches (Taylor et al., 2012, 2004; Ogwok et al., 2012).

Efforts are underway to use genetic engineering to reduce post-harvest deterioration of the storage roots, beginning with elucidating the physiological steps involved in the process (Taylor et al., 2004). The reduction and control of reactive oxygen species is a main focus of these efforts (Sayre et al., 2011).

Efforts to create herbicide-tolerant cassava varieties through genetic engineering are ongoing (Fermont et al., 2009). Herbicide tolerance is a trait perceived to be of particular value for industrial-scale cassava production (Taylor et al., 2004); however, herbicide-tolerant cassava might also reduce the high labour costs of manual weeding for smallholder farmers.

Other traits

There is increasing interest in the development of cassava as an industrial crop, specifically in the use of cassava in the production of biodegradable plastics. Research is underway to produce plastic precursors, such as polyhydroxyalkanoates in cassava (Fregene and Puonti-Kaerlas, 2002; Taylor et al., 2004; Lebot, 2009).

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Chapter 4.

Common bean (*Phaseolus vulgaris*)

*This chapter deals with the biology of common bean (*Phaseolus vulgaris*). It contains information for use during the risk/safety regulatory assessment of genetically engineered varieties intended to be grown in the environment (biosafety). It includes elements of taxonomy, centres of origin and distribution, crop production and cultivation practices, morphological characters, reproductive biology, genetics, hybridisation and introgression, interactions with other organisms, pests and pathogens, and biotechnological developments.*

This chapter was prepared by the OECD Working Group on the Harmonisation of Regulatory Oversight in Biotechnology, with Brazil as the lead country and the collaboration of the ILSI Research Foundation, Center for Environmental Risk Assessment (CERA). It was initially issued in December 2015. Production data from FAOSTAT have been since updated.

Species or taxonomic group

Classification and nomenclature

The scientific name of common bean is *Phaseolus vulgaris* L. (ITIS, 2014). The common bean is a member of the legume family, and its taxonomic hierarchy is:

Order Fabales

Family Fabaceae

Genus *Phaseolus* L.

Species *Phaseolus vulgaris* L.

Common synonyms are French bean, haricot bean, salad bean, snap bean, string bean, *frijoles* (Spanish), *feijão* and *feijoeiro* (Portuguese for the seed and the plant, respectively), and *mharagwe* (Swahili) (Purseglove, 1968; Wortmann, 2006; Gepts and Debouck, 1991).

The genus *Phaseolus* is large, including approximately 80 cultivated and wild species, but *P. vulgaris* is the most widely cultivated species (Purseglove, 1968; Freytag and Debouck, 2002; Bailey, 1975; Porch et al., 2013). The most closely related species to *P. vulgaris* are *P. albescens*, *P. coccineus*, *P costaricensis*, *P. dumosus*, *P. parvifolius* and *P. persistentus* (Table 4.1.) (Chacón et al., 2007; Broughton et al., 2003; Bellucci et al., 2014; Delgado-Salinas, Bibler and Lavin, 2006). In addition to *P. vulgaris*, four other *Phaseolus* species are cultivated: *P. dumosus* (year bean), *P. coccineus* (scarlet runner), *P. acutifolius* (tepary bean) and *P. lunatus* (lima bean) (Bellucci et al., 2014; Lioi and Piergiovanni, 2013).

Table 4.1. **Species closely related to *Phaseolus vulgaris***

Species	Geographic location
P. acutifolius	Mexico, southwestern United States
P. albescens	Western Mexico
P. coccineus	Guatemala, Honduras, Mexico
P. costaricensis	Eastern Costa Rica, western Panama
P. dumosus	Western Guatemala, Mexico
P. parvifolius	Southwestern United States, Guatemala, Pacific coast of Mexico and Central America
P. persistentus	Guatemala

Sources: Porch et al. (2013); Bellucci et al. (2014).

P. vulgaris belongs to the Fabaceae family, which comprises species displaying a wide variety of forms: trees, shrubs and herbs, including many with a climbing growth habit. Most species bear five-petaled flowers with a distinctive papilionaceous or butterfly-like shape. The flowers have a single large upright petal, flanked by two horizontal "wing" petals, and subtended by two petals at the bottom of the flower, partially or completely joined to form a boat-like "keel." Flowers typically have ten stamens, nine of which may form a tube surrounding the ovary and one that is separate from the others and positioned above the ovary, although there are variant stamen configurations in some species. The fruit of Fabaceae species is the legume – a single-carpelled pod of various shapes and sizes, bearing from one to many seeds. In many species the pod splits, either along one or both edges, known as the placental and central sutures, to release the seeds (Wortmann, 2006).

P. vulgaris shares many of the features characterising the family, but two features distinguish the entire *Phaseolus* genus from the rest of the family: the keel of the flower terminates in a coil, having from one to two turns (Bailey, 1975; Purseglove, 1968; Gentry, 1969), and uncinate hairs are present on both vegetative and reproductive structures of the plant (Freytag and Debouck, 2002).

The wild ancestor of *P. vulgaris* has been referred to as the same species (Gentry, 1969); as a variety of domesticated common bean, *P. vulgaris* var. *mexicanus* (Delgado-Salinas et al., 1988); as a separate species, *P. arborigineus* (Brücher, 1988); and as a subspecies, *P. vulgaris* subsp. *arborigineus* (Gentry, 1969).

Description

Common bean is the most commonly consumed legume worldwide, and it is the most important legume produced for direct human consumption, with a commercial value exceeding that of all other legume crops combined (Broughton et al., 2003; Porch et al., 2013; Graham and Vance, 2003). Although low in methionine and cysteine, the dried seeds, or "pulses", of *P. vulgaris* are an important source of dietary protein for millions of people throughout the tropics, supplementing those amino acids lacking in diets based on maize, rice or other cereals (Broughton et al., 2003; Wortmann, 2006). Beans are an especially valuable source of the amino acids lysine and tryptophan; the minerals iron, copper and zinc; and beneficial phytochemicals, antioxidants and flavonoids (FAO, 1999).

Dry beans are typically processed before consumption, usually by cooking in water, but some beans are consumed after roasting or after milling into flour (Tohme et al., 1995; Siddiq and Uebersax, 2012; FAO, 1999). Immature seed pods, called snap beans, are consumed as vegetables in some regions, and straw from the plants is used as forage (Purseglove, 1968; Broughton et al., 2003). The leaves of some specially selected varieties are consumed as a vegetable, usually when better quality food is not available (Wortmann, 2006).

In developing countries in Latin America and Africa, most beans are produced by smallholder farmers (Broughton et al., 2003), and a significant portion of the crop is consumed on-farm, so it is difficult to accurately estimate global production. The widespread practice of producing beans through intercropping also leads to an overestimation of the total area planted and an underestimation of global yields (Akibode and Maredia, 2011). The FAO reported that total dry bean production in 2014 was over 26 million tonnes (FAO, 2014), although this number includes other bean species as well, and possibly other minor food legumes.

Due to extensive plant-breeding efforts, *P. vulgaris* comprises numerous cultivars with a wide range of morphological and agronomic characteristics, including differences in seed size and colour as well as growth habit (Purseglove, 1968; Singh et al., 1991). One of the most commonly selected traits is determinate growth, which is associated with reduced branching, shorter and fewer internodes, reduced twining, insensitivity to day length, and most importantly, an increased allocation of biomass to reproductive growth (Kwak et al., 2012; Singh and Schwartz, 2010). Specific agronomic circumstances also favour the use of varieties with a determinate growth habit: they are better adapted to shorter growing seasons because they mature earlier; they produce pods over a shorter, more consistent period of time, which simplifies the harvest of green beans; and determinate varieties are more amenable to mechanised cultivation and harvest

(Kwak et al., 2012). Determinate and indeterminate growth habits are shown in Figure 4.1.

Figure 4.1. **Differences in growth habit in common bean: Determinate (left); indeterminate (right)**

Note: The arrows mark trifoliolate leaves, replaced by primary bracts in the determinate variety. The main stem is thus replaced by a terminal inflorescence in the determinate variety, while the main stem continues to produce axillary racemes in the indeterminate variety.

Source: Courtesy D.G. Debouck, CIAT.

There are also twining or climbing cultivars of *P. vulgaris* with indeterminate growth habit as well as many cultivars with a partially erect and partially trailing intermediate growth habit (Purseglove, 1968; Singh et al., 1991), although they are less frequently grown than the determinate cultivars. Prostrate to semi-climbing indeterminate varieties are favoured in cool, highland areas, with short day length (Singh and Schwartz, 2010). Typically, the length of the main stem of the plant is positively correlated with the number of nodes per stem and the number of seed pods produced (García et al., 1997).

Other traits selected as a result of the domestication of *P. vulgaris* are increased pod size and fleshiness, reduced pod dehiscence, larger seeds and increased permeability of the seeds to water (Gentry, 1969; García et al., 1997; Singh et al., 1991).

Cultivated *P. vulgaris* has a taproot-based root system with lateral roots typically located within the top 15 cm of soil. The roots are colonised by *Rhizobium* bacteria, resulting in irregular root nodules (Purseglove, 1968).

The stems are typically hairy, with the length and density of the hairs dependent on the cultivar. However, short, hooked hairs (uncinate hairs) are always present on the younger portions of the stems (Debouck and Hidalgo, 1986; Singh et al., 1991; Lackey, 1981; Freytag and Debouck, 2002). The hairs have a role in both disease and insect resistance. There is evidence that the hairs interrupt the production of fungal spores, thereby reducing secondary inoculum (e.g. bean rust, *Uromyces appendiculatus*) and can physically wound insects (such as leafhoppers, *Empoasca fabae*), resulting in reduced predation (Mmbaga and Steadman, 1992; Pillemer and Tingey, 1978). When the climate is sufficiently warm to allow a semi-perennial growth habit, the stems of wild *P. vulgaris* can grow to a diameter of 1.5 cm and may develop a corky outer layer (Gentry, 1969).

The leaves are trifoliolate and alternate on the stems. The leaflets are entire and somewhat hairy, 8-15 cm x 5-10 cm, with small stipules (Purseglove, 1968; Wortmann, 2006). Leaflet shape differs among the cultivars, but leaflets generally have broad bases and pointed tips (Singh et al., 1991).

Flowers are borne on axillary or terminal racemes, in colours of white, pink or violet, depending on the cultivar. The bisexual flowers are keeled, and the keel terminates in a coil, with one to two turns (Purseglove, 1968; Bailey, 1975; Wortmann, 2006).

The seed pods are narrow, 8-20 cm x 1-2 cm, with up to 12 seeds per pod, but most varieties have 4-6 seeds. Seeds are produced in a wide variety of colours, depending on the cultivar (Purseglove, 1968; Wortmann, 2006), and the seeds vary considerably in size, with a range of 150-900 g per 1 000 seeds (Brink and Belay, 2006; Wortmann, 2006).

Wild *P. vulgaris* differs from the cultivated types in several characteristics. The plants are typically indeterminate climbers with shorter main stems than the cultivated varieties. Main stem branches are more numerous, but with fewer nodes (Brücher, 1988; García et al., 1997; Delgado-Salinas et al., 1988; Gentry, 1969). A twining growth habit helps the plant to better compete for sunlight with forest vegetation than a shrubby determinate habit (Kwak et al., 2012; Gentry, 1969). Flowers, seed pods and seeds of the wild species are more numerous; pods and seeds are smaller; and the pods have a dehiscence slit near the pedicel and are explosively dehiscent (Brücher, 1988; García et al., 1997; Delgado-Salinas et al., 1988). The wild species has a much longer flowering period than cultivated varieties, and flowers can be produced up to the first killing frost (Brücher, 1988).

Physiological differences have also been identified between the cultivated and wild species. For example, nitrogen use efficiency and carbon dioxide exchange rates were found to be higher in wild populations when compared to cultivated landraces (Porch et al., 2013).

Geographic distribution, ecosystems, cultivation and management practices, centres of origin and diversity

Geographic distribution

Wild common bean populations were first documented in Guatemala in 1947 (McBryde, 1947), and they occur from northern Mexico to northern Argentina. However, the distribution is not continuous through that region, due to climatic variations unfavourable to the species, that is, regions with excessive rainfall or elevations below 700 metres or above 3 000 metres (Chacón et al., 2007; Chacón, Pickersgill and Debouck, 2005; Broughton et al., 2003). Habitat destruction throughout the species' range has accelerated the interest in identifying and preserving ancestral varieties (Debouck et al., 1993).

Wild common bean occurs from northern Mexico (Acosta-Diaz et al., 2015) to northwestern Argentina and distinct differences in both morphological characteristics and molecular markers have been identified in the northernmost and southernmost populations (Singh et al., 1991; Freyre et al., 1996). The climate where common bean originated is sub-tropical to temperate, with defined wet and dry seasons, and bean prefers regions with moderate rainfall, rather than dry regions or areas with excessive rain (Beebe et al., 2014). Bean plants cannot tolerate frost, or elevations above 3 000 metres, but they can grow as annuals in temperate climates and as annuals or short-lived

perennials in tropical climates (Purseglove, 1968; Gentry, 1969). Excessive temperatures cause flowers to abscise, and low temperatures delay pod production and can result in empty pods (Liebenberg, 2009). Common bean prefers well-drained, sandy clay or sandy loam soils, with balanced fertility and moderate acidity pH 5.8-6.5 (Liebenberg, 2009).

Ecosystems where common bean occurs natively and has naturalised

Having evolved in areas where taller vegetation limits the sunlight that reaches the forest floor, wild bean grows as a vigorous vine that enables it to effectively compete for sunlight (Beebe et al., 2014), a characteristic that enables wild bean to exploit disturbed sites, using other pioneer species as climbing support (Brücher, 1988; Delgado-Salinas et al., 1988).

Cultivated varieties of bean do not tend to persist as feral populations in regions outside the species' native range. Genetic analyses of individual bean plants selected from feral populations and cultivated varieties indicate that the cultivated varieties have been derived from feral populations, rather than the other way around (Porch et al., 2013; Beebe et al., 1997; Toro Ch. and Ocampo, 2004).

Cultivation and management practices

P. vulgaris is planted in pure stands of single landraces, as mixed plantings of several landraces, and intercropped with maize, sweet potatoes, cotton, coffee and other crops. It is common for farmers to freely exchange their landraces (Zizumbo-Villarreal et al., 2005; Wortmann, 2006). Typically, beans planted for vegetable use are planted in monoculture (Singh and Schwartz, 2010; Wortmann, 2006). Because bean varieties consumed as a vegetable produce pods in as little as two months, rotations with other crops is a common practice (Purseglove, 1968; Broughton et al., 2003).

Whether a farmer plants one or two bean crops per year is determined largely by rainfall patterns. In tropical regions having a bimodal pattern, two plantings per year are possible, but in more temperate climates with a single rainy season, only one crop is planted (Beebe et al., 2014).

Seed is either sown in rows or broadcast, with seeding rates of 150 000-400 000 seeds per hectare. When intercropped, beans are sown at a lower rate (Wortmann, 2006). Examples of intercropping with coffee and maize are shown in Figures 4.2 and 4.3. Bush-type varieties are typically planted at higher densities (30-90 cm x 15-30 cm) than pole-type varieties (hills 30-120 cm apart, 3-6 plants per hill). Even within the type, planting densities vary widely, depending on local practice and degree of mechanisation (Purseglove, 1968; Liebenberg, 2009; Wortmann, 2006); however, increasing the planting density generally increases yields (Russo and Perkins-Veazie, 1992).

In developed countries, where mechanised cultivation is practiced, row planting is common, using inter-row distances of 75-90 cm, depending on the variety (Liebenberg, 2009). Greater degrees of mechanisation require varieties with more uniform growth habit and maturation time (FAO, 1999). More widely spaced rows facilitate cultivation, while planting more closely spaced rows results in larger plants, more numerous pods and higher yields, depending on the environmental conditions (Goulden, 1975). However, close spacing can increase disease incidence (Sandoval-Avila et al., 1994).

Beans are typically planted on level land, but sowing on hills or ridges may be practiced in areas with heavy soils or where the water table is high (Wortmann, 2006). Soil preparation in developed countries includes cultivation and the application of any needed fertiliser (Purseglove, 1968). Due to the variable effectiveness of nitrogen fixation

by common bean, nitrogen content of the soil is typically supplemented in commercial production (Liebenberg, 2009). Phosphorus and potassium deficiencies severe enough to cause yield losses are not common in developed countries (Liebenberg, 2009).

Figure 4.2. **Common beans intercropped with coffee**

Source: Courtesy Embrapa.

Figure 4.3. **Common beans intercropped with maize**

Source: Courtesy Embrapa.

Seed germination needs a minimum soil temperature of 12°C, with an optimum temperature of 22-30°C. Depending on the variety, flowering begins four to six weeks after sowing (Wortmann, 2006). High night temperatures during anthesis can cause flowers to abort and reduce seed set (Russo and Perkins-Veazie, 1992). Determinate bean varieties face greater competition from weeds, because weeds may overgrow the crop, so weed control, especially in the early establishment of the crop, is important (Liebenberg, 2009; Wortmann, 2006).

Harvest times depend on the use of the crop. For snap beans consumed as a vegetable, harvest begins two to four weeks after flowering (seven to eight weeks after sowing). For dry beans, harvest occurs when the pods have turned yellow and the seeds have matured (Purseglove, 1968; Wortmann, 2006). Seed filling takes from three to seven weeks. Although seed maturity occurs when the moisture content is approximately 50%, harvesting does not typically occur until the seeds dry down to 15-16%. Significant losses can occur post-harvest if plants are left to dry excessively before moving them to the threshing area, because seed pods may open spontaneously and drop seeds on the ground (FAO, 1999). Additionally, allowing seeds to lose additional moisture prior to harvest increases the risk of split seeds, which is a problem in commercial production (Liebenberg, 2009).

Physiological and biochemical ripening continues even after harvest, and some of these processes can impair the quality of the harvest. The beans develop a brown discolouration and off-flavours as well as textural defects that appear after cooking – a condition called "bin burn." The potential for bin burn and cooking defects is both genetically and environmentally determined, but allowing the beans to dry to 11-12% moisture content and storing seed under cool conditions generally helps preserve seed quality (FAO, 1999).

Plants may be hand harvested and threshed in the case of smallholder farms, or in the case of commercial production, the harvest and threshing processes may be mechanised (Liebenberg, 2009). In some regions, seeds are sorted by variety while in other areas, seeds of various varieties with similar cooking requirements are commingled and consumed as a mixture (Wortmann, 2006; FAO, 1999).

Inputs also vary depending on the region. Beans are produced successfully without irrigation in regions receiving from 25 cm to over 40 cm of rainfall during the growing season (Wortmann, 2006). Commercial production in developed countries and in arid subtropical regions may use irrigation to supplement natural rainfall (Purseglove, 1968). In developing countries, beans may be grown with no mineral fertilisers or manure, while in developed countries mineral fertilisers are used routinely.

In developing countries, significant yield losses from disease, insect pests, low soil fertility and abiotic stresses are common (Broughton et al., 2003). Low soil phosphorus is a major constraint to common bean production, especially when grown by resource-poor farmers in tropical and subtropical regions, where acidic soils tend to be phosphorus deficient (Beebe, 2006; Beebe et al., 2014; Graham and Vance, 2003; Porch et al., 2013). In addition, many farmers in developing countries treat beans as a low-input crop, choosing to allocate scarce resources to other crops, such as cereals (Akibode and Maredia, 2011). Because of these limitations, bean yields in developed countries are typically several times that of yields in developing countries (Porch et al., 2013).

Improvements in heat and drought tolerance have the potential to significantly increase bean yields in the majority of regions where beans are grown (Porch et al., 2013). However, breeding efforts to create bean varieties able to cope with abiotic and biotic stresses are hampered by a lack of available genes for stress resistance. Identifying new varieties is made even more difficult by the need for breeders to meet consumer requirements for what are often very specific bean size, taste, colour and quality characteristics (Singh and Schwartz, 2010). The tepary bean, *P. acutifolius*, is thought to be a promising source of genes for increasing tolerance to abiotic stresses, such as high temperature, drought and high salinity (Porch et al., 2013).

Centres of origin and diversity

Although 200 years ago it was believed that common bean originated in Asia, a large body of evidence indicates that *P. vulgaris* originated in the New World (Kaplan and Lynch, 1999; Gepts and Debouck, 1991). Archaeological records indicate that the species originated and was first domesticated as early as 5 000 B.C. (Purseglove, 1968; Bitocchi et al., 2013, 2012), although there is evidence for a more recent origin in Mesoamerica (Kaplan and Lynch, 1999). Multi-locus sequence data have indicated that the domestication of common bean was initiated 8 000 years ago (Mamidi et al., 2011).

Polymorphisms among cultivated varieties and molecular markers, such as isozymes and variants of the seed protein phaseolin, indicate that there may have been at least two independent centres of domestication in Central and South America (Purseglove, 1968; Singh et al., 1991; Bitocchi et al., 2013, 2012; Chacón, Pickersgill and Debouck, 2005; Bellucci et al., 2014; Kaplan and Lynch, 1999; Freyre et al., 1996), resulting in the Middle American and the Andean gene pools (Acosta-Gallegos, Kelly and Gepts, 2007; Brücher, 1988; Kwak and Gepts, 2009; Angioi et al., 2010). Some evidence indicates that these two gene pools had already diverged before domestication efforts began (Brücher, 1988; Delgado-Salinas et al., 1988). The South American types tend to have seeds and leaves of larger size than the Central American varieties (Wortmann, 2006).

Cultivated common bean were developed from wild common bean, and domestication has introduced several agronomically useful traits: indeterminate and bush types; increased leaf, pod and seed size; and suppression of pod dehiscence and seed dormancy. Vast diversity of seed size, shape and colour has also resulted from domestication (Singh et al., 1991; Broughton et al., 2003). Crop earliness has been enhanced by

selecting for photoperiod insensitivity (White and Laing, 1989). Domestication of the common bean has also resulted in a significant reduction in genetic diversity, compared to the species in the wild (Bitocchi et al., 2013; Chacón, Pickersgill and Debouck, 2005).

Spanish and Portuguese explorers eventually brought *P. vulgaris* (Figure 4.4) to Europe in the 16th century (Purseglove, 1968), and Portuguese traders are believed to have then brought beans to Africa, where they spread from the highland areas of Central Africa to the rest of the continent (Wortmann, 2006).

Figure 4.4. **Wild species of common bean (*P. vulgaris*)**

Source: Courtesy Dr. Ismael Hernández, INIFAP-México.

Reproductive biology

Generation time and duration

Common bean can grow as annuals in temperate climates and as annuals or short-lived perennials in tropical climates (Purseglove, 1968; Gentry, 1969). The number of days to seed maturity varies widely, from 50 to more than 250 days, and it is dependent on the cultivar, its photoperiod response and the environmental conditions (Singh et al., 1991; Sandoval-Avila et al., 1994; White and Laing, 1989).

Reproduction

Floral biology

Flowers of wild *P. vulgaris* are generally purple, pink or white (Gentry, 1969) (Figure 4.5). The floral structure of *P. vulgaris* contributes to the high rate of self-pollination: anther dehiscence and stigma receptivity occur at the same time, before the flower is fully open, and the anthers and stigma are positioned near one another at the time of anther dehiscence and stigma receptivity (Webster, Tucker and Lynch, 1977).

Bracts on the rachis of the inflorescences are persistent (Lackey, 1981), and the size and shape of the bracteoles are distinguishing characteristics of bean cultivars (Singh et al., 1991).

Figure 4.5. **Flower of *Phaseolus vulgaris*, showing coiled keel**

Source: Courtesy D.G. Debouck, CIAT.

Pollination and pollen dispersal

The pollen grains of common bean have a diameter of approximately 30 micrometres. They are spherical to triangular and tricolporate in shape, with a reticulate exine (Ferguson, 1984). Little is known about the longevity of bean pollen (Andersson and de Vincente, 2010).

Common bean is regarded primarily as a self-pollinating species, due to floral morphology (Purseglove, 1968; Singh et al., 1991). However bumble bees, carpenter bees and honeybees have been identified as potential pollen carriers between cultivated bean plants. These species, as well as other insects such as thrips, are responsible for the low frequencies of outcrossing observed between bean varieties grown in close proximity (Ferreira et al., 2006; Free, 1966; Proctor, Yeo and Lack, 1996; Faria, Carneiro and Aragão, 2010). Published reports indicate that the outcrossing frequency approaches zero when bean plants are separated by three to ten metres (Ferreira et al., 2006; Faria, Carneiro and Aragão, 2010), but outcrossing rates are dependent on both the bean genotype and the environmental conditions (Wells, Isom and Waines, 1988; Ibarra-Perez, Ehdaie and Waines, 1997). Intervarietal cross-pollination would also depend on synchrony of flowering (Ferreira et al., 2000). Examples of standard isolation distances established for the production of certified bean seed are three metres (Canada) (CSGA, 2013), five metres (Common Market of Eastern and Southern Africa and India) (Indian Ministry of Agriculture, 2013; COMESA, 2014) and zero metres or a distance adequate to prevent mechanical mixture (United States) (AOSCA, 2009).

Seed production, and natural dispersal of fruits or seeds

The number of days for seed maturity varies widely, from 50 to more than 250 days, and is dependent both on the cultivar and the environmental conditions (Singh et al., 1991).

Seed dispersal is minimal when beans are grown as snap beans for vegetable use, because the pods are harvested before the seeds are mature. Modern bean varieties are selected for non-dehiscence of mature pods, so few seeds are dispersed via this route, and any dispersal would occur over only short distances (Gentry, 1969; Acosta-Gallegos,

Kelly and Gepts, 2007; García et al., 1997). Birds are known to consume immature seeds while still in the developing pods, but there is little evidence that animals disperse mature seeds, probably due to their toxicity (Debouck et al., 1993).

Seed viability, dormancy and natural seed banks

True seed dormancy in common bean is rarely encountered (Acosta-Gallegos, Kelly and Gepts, 2007; Westphal, 1974); however, seeds of wild bean and some cultivars have a hard seed coat that is only partially permeable to water, thereby inhibiting germination (Brücher, 1988; Freyre et al., 1996). As a result, seeds can remain ungerminated in the soil for two years (Purseglove, 1968). Breeding efforts have had success in increasing the permeability of the seed coat as a means of ensuring more uniform germination (García et al., 1997; Singh et al., 1991; Bellucci et al., 2014).

Asexual propagation (apomixis, vegetative reproduction)

Bean is propagated primarily using seeds, although it is possible to propagate bean vegetatively, using stem cuttings (Wortmann, 2006; Brink and Belay, 2006).

Genetics

Both cultivated and wild forms of the species are diploid (2n = 22), and the two forms hybridise readily (Delgado-Salinas et al., 1988; Singh et al., 1991).

Crosses between the Middle American and Andean gene pools are easily accomplished, although differences in flowering time can make crossing difficult (Porch et al., 2013). It has been noted that divergences between the two gene pools may make recovery of progeny more difficult than with crosses within the two pools, and occasionally crosses result in dwarfism or lethality (Acosta-Gallegos, Kelly and Gepts, 2007; Singh and Schwartz, 2010). This hybrid weakness is thought to be due to semi-dominant alleles of two "dosage-dependent lethal" (DI) genes. Depending on the heterozygosity of these two genes, hybrids between the two gene pools may exhibit complete lethality, lethality at high temperatures or only sublethal symptoms (Table 4.2) (Koinage and Gepts, 1992).

Table 4.2. **Hybrid weakness in wild *P. vulgaris***

	Homozygous DI2 locus	Heterozygous DI2 locus
Homozygous DI1 locus	Lethal	Sublethal
Heterozygous DI1 locus	Sublethal	Abnormal phenotype at high temperature

Hybridisation and introgression

Natural crosses between common bean and other *Phaseolus* species are inhibited by a variety of incompatibility mechanisms, such as incomplete chromosome pairing, sterility of F_1 hybrids and embryo abortion (Broughton et al., 2003). Other barriers, such as photoperiod sensitivity and flowering time, have also been noted as limiting opportunities for interspecific crossing without human intervention (Porch et al., 2013). However, wild-collected plants representing hybrids of *P. vulgaris* x *P. coccineus* have been reported (Escalante et al., 1994).

Experimental crosses have been attempted between *P. vulgaris* and several closely related species, such as *P. coccineus*, *P. dumosus*, *P. costaricensis*, *P. acutifolius*, *P. parvifolius*, *P. filiformis* and *P. angustissimus*, to take advantage of disease and insect

resistance and abiotic stress tolerance traits that these species possess (Acosta-Gallegos, Kelly and Gepts, 2007; Escalante et al., 1994; Schwartz and Singh, 2013; Beebe et al., 2014). However due to partial incompatibility, viable offspring from such crosses may require embryo rescue, and hybrids frequently exhibit dwarfism and partial or complete sterility (Broughton et al., 2003; Brücher, 1988; Acosta-Gallegos, Kelly and Gepts, 2007; Singh and Schwartz, 2010). Using *P. vulgaris* as the female parent may reduce the need for embryo rescue (Porch et al., 2013).

Data indicate that under the right environmental conditions, cultivated *P. vulgaris* plants can pollinate nearby wild *P. vulgaris* plants, resulting in fertile hybrids and the potential for domestication traits to introgress into wild populations (Zizumbo-Villarreal et al., 2005; Delgado-Salinas et al., 1988; Freyre et al., 1996). Conversely, when the wild plants act as the male parent, gene flow to cultivated varieties can also occur, although at much lower frequency than when the cultivated variety acts as the male parent (Papa and Gepts, 2003). These hybrids, when harvested by the farmer and replanted, increase the genetic diversity of regional landraces and are considered to have a positive impact on the cultivated species (Zizumbo-Villarreal et al., 2005; Beebe et al., 1997). However, data indicate that, in spite of the possibilities for hybridisation between feral and cultivated populations of bean, the two populations generally remain strongly differentiated (Papa and Gepts, 2003).

Manual crosses between cultivated bean varieties and wild *P. vulgaris* are easily made, resulting in viable, fertile F_1 offspring (Brücher, 1988). There is evidence that under certain conditions, low to moderate levels of natural outcrossing with the wild species can occur (Singh et al., 1991; Kwak, Kami and Gepts, 2009; Ibarra-Perez, Ehdaie and Waines, 1997), possibly mediated by insect pollinators, such as bumblebees (Brücher, 1988; Delgado-Salinas et al., 1988). The high level of homozygosity in wild populations indicates that outcrossing is generally a rare occurrence (Kwak, Kami and Gepts, 2009).

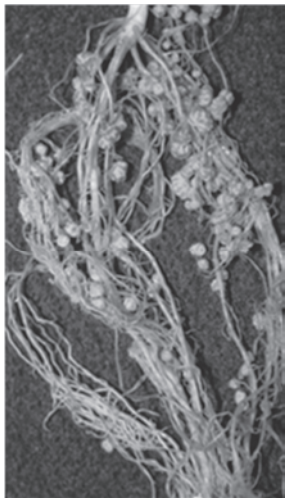
General interactions with other organisms (ecology)

Like other legumes, *P. vulgaris* associates with *Rhizobium* bacteria in the soil, which form root nodules (Figure 4.6). Through nitrogenase activity, the bacteria within the nodules fix atmospheric nitrogen to form ammonia, which the bean plant uses as a nitrogen source, reducing the need for externally applied fertilisers (Ramos et al., 2003). However, the nitrogen-fixing capacity of *P. vulgaris* varies by variety and is generally less than that of other agronomically important legumes, such as soybeans, which tend to have larger root nodules with higher nitrogenase activity (Isoi and Yoshida, 1991; Hardarson et al., 1993). *P. vulgaris* roots are colonised by a wide range of native *Rhizobium* species and strains, some of which have little or no nitrogenase activity (Isoi and Yoshida, 1991; Ribeiro et al., 2013; Vásquez-Arroyo et al., 1998), and this may be one of the reasons for reduced nitrogen-fixing capacity.

Several environmental factors present in regions where beans are commonly grown, such as drought, flooding and either high or low temperatures, impact nitrogen fixation. *Rhizobium* populations, nodulation, ammonium assimilation and nitrogenase activity are all reduced under these conditions (Beebe et al., 2014; Devi et al., 2012; Hungria and Kaschuk, 2014; Ramos et al., 2003; Vásquez-Arroyo et al., 1998; Graham, 1981). Low soil phosphorus and manganese levels as well as low soil pH are also associated with sub-optimal nitrogen-fixing capacity (Graham and Vance, 2003; Ramos et al., 2003; Wortmann, 2006; Graham, 1981). *Rhizobium*-mediated nitrogen fixation can be enhanced

by increasing planting density (Graham, 1981), but there is evidence that intercropping may inhibit nitrogen fixation by increasing competition for water and soil nutrients (Graham, 1981).

Figure 4.6. ***Rhizobium* nodules on the roots of common bean**



Source: Courtesy Embrapa.

Planting beans in soil where they have not been grown before can also result in poor nitrogen fixation, due to insufficient *Rhizobium* in the soil to initiate nodulation (Wortmann, 2006). However, smallholder farmers do not typically use *Rhizobium* inoculants prior to planting beans (Graham, 1981). In addition, the use of some pesticides, such as fungicides that are toxic to *Rhizobium*, can inhibit root nodulation.

Human health

Information on common bean and its major products, as well as food and feed safety considerations including composition in terms of key food and feed nutrients, anti-nutrients and other constituents, have been summarised by the OECD in another document issued in the Series on the Safety of Novel Foods and Feeds (OECD, 2015). Therefore, it is not included here.

Annex 4.A1. Common pests and pathogens

Common bean is susceptible to many pests and diseases, although endemic pests and diseases vary with geographic location. In combination with sub-optimal growing conditions, common in the low-input scenarios used in developing countries, pests and diseases may act synergistically to cause significant, and sometimes total, yield losses (Graham and Vance, 2003; Singh and Schwartz, 2010). The value of harvested seed is reduced due to decreased germination and poor quality (Singh and Schwartz, 2010).

Pests

There are several serious insect pests that attack the common bean, depending on the geographic location, but predation by a wide range of arthropods – aphids, beetles, caterpillars, leafhoppers, whiteflies, mites and thrips – is seen worldwide (Cardona, 1989; Karel and Autrique, 1989; Quintela, 2009). Post-harvest damage from rodents is less of a problem because uncooked dry beans are toxic to mammals (FAO, 1999). Typically, chemical pesticides are used more commonly in the commercial production setting, rather than by smallholder farmers (FAO, 1999). Table 4.A1.1 summarises the main arthropods identified as potential pests for common bean.

Table 4.A1.1. **Arthropod pests of common bean**

Scientific and common name	Types of damage	Control methods	Resistant species
Storage pests			
Zabrotes subfasciatus Mexican bean weevil	Damage to mature seed in storage	Mixing seeds with ash, sand or lime; refrigerated storage; coating with edible oil; fumigation	*P. vulgaris*
Acanthoscelides obtectus Bean weevil, bean beetle	Damage to mature seed in storage	Mixing seeds with ash, sand or lime; refrigerated storage; coating with edible oil; fumigation	*P. vulgaris*
Seedling-attacking pests			
Delia pratura Seedcorn maggot	Larvae feed on bean seeds or seedlings	Cultural practices (shallow planting in warm, moist soil) seed	*P. vulgaris*
Elasmopalpus lignosellus Lesser cornstalk borer	Larvae enter the stem just below soil surface and tunnel upwards	Heavy irrigation and proper land preparation and weed control	No good resistance
Agrotis ipsilon, *Spodoptera* spp. Cutworms	Larvae cut stems of young seedlings. Older plants can be damaged by stem girdling.	Proper land preparation and weed control	No good resistance
Teratopactus nodicollis	Larvae cause damage at germination, emergence and during early vegetative growth. When larvae feed on the radicle and hypocotyl, the seedlings die before emergence.	Cultural practices (proper land preparation, weed control, increasing planting rate)	No good resistance
Ophiomyia phaseoli, *O. specerella* Bean fly, bean stem maggot	Feed on stem at seedling stage	Seed and seedling treatments with systemic insecticides	*P. vulgaris*, *P. coccineus*
Leaf-feeding pests			
Diabrotica spp., *Cerotoma* spp. Chrysomelids	Larvae damage roots and roots nodules, adults feed on foliage and are vectors of important viral diseases	Yellow traps; neem oil as antifeedant agent	No good resistance

Table 4.A1.1. **Arthropod pests of common bean** (*continued*)

Scientific and common name	Types of damage	Control methods	Resistant species
Liriomyza spp. Leafminers	Larvae damage leaves by making serpentine tunnels while feeding on leaf palisade tissues	The insect is usually controlled by natural enemies	No good resistance
Omiodes indicate Webworm	Larvae weave leaves together and feed on the parenchyma	The insect is usually controlled by natural enemies	No good resistance
Urbanus proteus Bean leafroller	Larvae fold the leaf margin and feed within the fold	Chemical control is seldom required	No good resistance
Chrysodeixis (=Pseudoplusia) includes Soybean looper	Larvae feed on underside of the leaves, avoiding the veins of the leaves, leaving a transparent appearance on parts of the leaf	*Bacillus thuringiensis* sprays, *Trichogramma* releases	No good resistance
Helicoverpa armigera	Larvae feed on leaves and pods	*Bacillus thuringiensis* and *Baculovirus* sprays, *Trichogramma* releases	No good resistance
Epilachna varivesta Mexican bean beetle	Adults and larvae feed on leaves. Stems and pods can also be damaged when populations are high.		*P. vulgaris*
Ootheca spp. Foliage beetles	Feed on leaves during pre-flowering period; virus vector	Crop rotation, intercropping, resistant cultivars	No good resistance
Epinotia aporema			No good resistance
Piercing and sucking pests			
Empoasca spp. Leafhoppers	Desiccation and necrosis of leaves; transmission of viral diseases	Intercropping with corn; *Zoophthora* spp. epizootics	*P. vulgaris*
Aphis fabae, A. craccivora Aphids	Sucks plant sap from leaves and stems at seedling stage and from pods; virus vector	Crop rotation, intercropping, resistant cultivars	*P. vulgaris*
Thrips palmi, *T. tabaci*, *Frankliniella occidentalis*, *F. schultzei*, *Caliothrips brasiliensis*, *Megalurothrips sjostedti* Thrips	Damage to leaves and growing tips	Crop rotation, intercropping resistant cultivars	*P. vulgaris*
Bemisia tabaci, Trialeurodes vaporariorum Whitefly	Adults and nymphs suck sap from leaves; main damage as virus vector	Crop rotation, intercropping, resistant cultivars	*P. vulgaris*
Polyphagotarsonemus latus, *Tetranhychus urticae* Mites	Suck sap from the lower surfaces of leaves	Insecticide sprays for egg and nymph control	No good resistance
Pod-attacking pests			
Apion godmani Bean pod weevil	Damage to immature pods and seeds	Bean-corn associations	*P. vulgaris*
Maruca vitrata, *Spodoptera* spp., *Etiella zinchenella* Pod borer	Larvae feed on developing seeds and expel frass into pod	*Bacillus thuringiensis* sprays	No good resistance
Clavigralla spp. Spiny bug	Suck sap from green pods, causing premature drying	Insecticide sprays	No good resistance
Neomegalotomus simplex	Adults and nymphs suck sap from green pods	Insecticide sprays	No good resistance
Nezara viridula, *Euschistus heros*, *Piezodorus guildini*, *Thyanta perditor, Edessa meditabunda*, *Chinavia* spp. Stink bugs	Suck sap from developing pods, thereby shriveling pods and seeds. Cause loss of yield and reduce germination of surviving seeds.	Insecticide sprays	No good resistance

Sources: Porch et al. (2013); Purseglove (1968); Miklas et al. (2006); Sanchez-Arroyo (2014); Wortmann (2006); FAO (1999); Cardona (1989); Karel and Autrique (1989); Quintela (2009).

Diseases

The main fungal diseases affecting common bean are listed in Table 4.A1.2, bacterial diseases in Table 4.A1.3 and viral diseases in Table 4.A1.4.

Table 4.A1.2. **Fungal diseases of common bean**

Name	Disease symptoms	Control methods	Resistant species
Thanatephorus cucumeris Web blight	Brownish, irregular lesions on pods; under humid conditions, mycelia will cover pods	Application of fungicides, planting disease-free seed	*P. vulgaris*
Colletotrichum lindemuthianum Anthracnose	Dark brown to black lesions affecting stems, pods and lower surfaces of leaves	Plant disease-free seed, application of fungicides, crop rotation	*P. vulgaris*, *P. coccineus*, *P. dumosus*
Sclerotinia sclerotiorum White mold	Destruction of the tissue, followed by superficial growth of white mycelia, under humid conditions. Seed-transmitted disease.	Application of chemical or biological pesticides, wide-row spacing, use of upright cultivars	*P. vulgaris*, *P. coccineus*, *P. dumosus*, *P. costaricensis*
Phoma exigua var. *diversispora*, *P. exigua* var. *exigua* Ascochyta blight	Red-brown lesions on leaves, stems, pods. Can cause rapid plant death.	Plant resistant varieties, plant clean seed, long crop rotations	*P. coccineus, P. dumosus*
Fusarium solani Fusarium root rot	Reddish-brown lesions on stems, lengthwise cracks that may extend down the main taproot, which decays	Good soil drainage, long crop rotations	No good resistance
Fusarium oxysporum Fusarium wilt	Yellowing and wilting of lower leaves, stunting	Plant resistant varieties	*P. vulgaris*
Rhizoctonia solani Rhizoctonia root rot	Damping off, oval, reddish-brown lesions on the hypocotyl, cankers on older stems	Fungicidal seed treatments, crop rotation	No good resistance
Uromyces phaseoli, *U. appendiculatus* Bean rust	Dry yellow to reddish spore masses on lower leaf surfaces and pods	Plant resistant varieties, fungicide applications	*P. vulgaris*
Phaeoisariopsis griseola Angular leaf spot	Grey to brown leaf lesions becoming necrotic; lesions may appear on stems and pods; pod lesions are oval and reddish-brown	Planting disease-free seed, fungicides, sanitation practices	*P. vulgaris*, *P. dumosus*, *P. coccineus*

Sources: Singh and Schwartz (2010); Schwartz and Singh (2013); Purseglove (1968); Kelly et al. (2003); Porch et al. (2013); Schmit and Baudoin (1992); Miklas et al. (2006).

Table 4.A1.3. **Bacterial diseases of common bean**

Name	Disease symptoms	Control methods	Resistant species
Xanthomonas campestris pv. *phaseoli* or *Xanthomonas axonopodis* pv. *Phaseoli* Common bean blight	Necrotic lesions on leaves, pods and seeds; seed-transmitted disease	Planting of disease-free seed, removal of disease reservoir plants in the field and the application of copper-based bactericides	*P. vulgaris*, *P. acutifolius*, *P. coccineus*
Pseudomonas syringae pv. *phaseolicola* or *Pseudomonas savastonoi* pv. *Phaseolicola* Halo blight	Brown necrotic spots surrounded by a light green halo, appearing on both leaves and stems. Infections can be systemic, and seeds may carry the disease.	Planting of disease-free seed, removal of disease reservoir plants in the field and the application of copper-based bactericides	*P. vulgaris*
Pseudomonas syringae pv. *Syringae* Bacterial brown spot	Brown lesions on both leaves and pods; seed-transmitted disease	Planting of disease-free seed, removal of disease reservoir plants in the field and the application of copper-based bactericides	*P. coccineus*

Sources: Liebenberg (2009); Singh and Schwartz (2010); Kelly et al. (2003); Porch et al. (2013).

Table 4.A1.4. **Viral diseases of common bean**

Name	Disease symptoms	Control methods	Resistant species
Bean common mosaic virus Potyvirus	Mosaic mottling of the leaves; vectored by aphids; seed-transmitted disease	Planting virus-free seed and using pesticides to control aphid populations	*P. vulgaris*
Bean golden mosaic virus Geminivirus	Yellow-green mosaic on leaves, stunted growth and distorted pods. Significant losses, as high as 100%.Vectored by whitefly (*Bemisia tabaci*).	Insecticide applications to control the vector	*P. vulgaris* (low level), *P. coccineus*
Bean common mosaic necrosis virus Potyvirus	Light green to yellow mosaic pattern on leaves, with puckering and rolling of the leaves	Plant resistant varieties; virus-free seed	*P. vulgaris*
Beet curly top virus Curtovirus	Strong down-cupping and puckering of leaves. Leaves are thickened and brittle and turn dark green. Plants are dwarfed. Vectored by leafhoppers.	Plant resistant varieties, virus-free seed; insecticide sprays to control leafhopper vectors	*P. vulgaris*
Bean yellow mosaic virus Potyvirus	Bright yellow to green mosaic pattern on leaves, cupping and wrinkling of leaves. Vectored by aphids.	Plant resistant varieties, virus-free seed, insecticide sprays to control aphid vectors	*P. vulgaris*

Sources: Singh et al. (2009); Singh and Schwartz (2010); Miklas et al. (2006); Bonfim et al. (2007); Aragão et al. (2013); Faria et al. (2014).

Annex 4.A2.
Biotechnological developments

Yield-limiting factors in common bean include insect predation, diseases and abiotic stressors. Biotechnological approaches to address these factors are the subject of numerous ongoing research efforts. Although the transformation and successful regeneration of common bean remains challenging (Veltcheva et al., 2005; Bonfim et al., 2007), bean has been successfully transformed by treating a variety of explants with *Agrobacterium tumefaciens* and via biolistic methods (Bonfim et al., 2007; Aragão and Faria, 2009; Faria et al., 2014; Faria, Carneiro and Aragão, 2010; Zhang, Coyne and Mitra, 1997; Kwapata, Nguyen and Sticklen, 2012).

Common bean has been transformed using marker genes: *GUS* (ß-glucuronidase) (Zhang, Coyne and Mitra, 1997), *bar* (Faria, Carneiro and Aragão, 2010), and *ahas* (Bonfim et al., 2007). The *bar* and *ahas* genes confer resistance to the herbicides phosphinothricin and imazapyr, respectively.

Bean has also been transformed to be resistant to the bean golden mosaic virus (Faria et al., 2014; Aragão et al., 2013; Bonfim et al., 2007; Aragão and Faria, 2009). Resistance was mediated using RNA interference, and the target of interference was the AC1 viral gene, which encodes a protein responsible for virus replication (Bonfim et al., 2007). In 2011, a transgenic bean event was approved for commercial cultivation in Brazil, which is resistant to bean golden mosaic virus (Calvalho et al., 2015).

Significant progress has been made on the sequencing of the bean genome, and approximately 80% of the genome has been sequenced and assembled (Schmutz et al., 2014).

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Chapter 5.

Cowpea (*Vigna unguiculata*)

*This chapter deals with the biology of cowpea (*Vigna unguiculata*). It contains information for use during the risk/safety regulatory assessment of genetically engineered varieties intended to be grown in the environment (biosafety). It includes elements of taxonomy, centres of origin and distribution, crop production and cultivation practices, morphological characters, reproductive biology, genetics and genome mapping, species/subspecies hybridisation and introgression, interactions with other organisms, human health considerations, common pests and pathogens, and biotechnological developments.*

This chapter was prepared by the OECD Working Group on the Harmonisation of Regulatory Oversight in Biotechnology, with Australia as the lead country. It was initially issued in December 2015. Updates have been made to the production data from FAOSTAT.

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is grown in tropical Africa, Asia, North and South America mostly as a grain, but also as a vegetable and fodder crop. It is favoured because of its wide adaptation and tolerance to several stresses. It is an important food source and is estimated to be the major protein source for more than 200 million people in sub-Saharan Africa and is in the top ten fresh vegetables in the People's Republic of China (hereafter "China").

In the English-speaking parts of Africa it is known as cowpea whereas in the Francophone regions of Africa, the name "*niébé*" is most often used. Local names for cowpea also include "*seub*" and "*niao*" in Senegal, "wake" or "bean" in Nigeria, and "luba hilu" in the Sudan. In the United States, it is typically referred to as blackeye beans, blackeye peas, crowder peas and southern peas. On the Indian subcontinent it is called "*lobia*" and in Brazil it is "*caupi*." In China it is called "long bean" or "asparagus bean".

Species or taxonomic group

Classification and nomenclature

Cowpea (*Vigna unguiculata* (L.) Walp.) belongs to the family *Fabaceae* (*Leguminosae* is also used as the family name with *Papilionoideae* as the subfamily), genus *Vigna*, and section *Catiang* (Verdcourt, 1970; Maréchal, Mascherpa and Stainier, 1978) (Table 5.1).

Table 5.1. **Classification of cowpea (*Vigna unguiculata* (L.) Walp.)**

Taxonomic placement	Scientific name
Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Order	*Fabales*
Family	*Fabaceae*
Sub-family	*Faboideae*
Tribe	*Phaseoleae*
Sub-tribe	*Phaseolinae*
Genus	*Vigna*
Section	*Catiang*
Species	*unguiculata*
Botanical varieties	1. *Vigna unguiculata unguiculata* var. *unguiculata* 2. *Vigna unguiculata unguiculata* var. *spontanea*

Annual cowpea has two botanical varieties (Table 5.1), the cultivated *Vigna unguiculata unguiculata* var. *unguiculata* and the wild form *V. u. u.* var. *spontanea*, both of which are inbreeding. *V. u. u.* var. *spontanea* is typically found mostly near the borders of cultivated cowpea fields and within them.

Cultivated cowpeas have been divided into five cultivar groups based mainly on pod, seed and ovule characteristics (Pasquet, 1999; 1998) (Table 5.2).

Unguiculata is the largest cultivar group. The cultivar group Sesquipedalis (variously known as "asparagus bean", "yardlong bean", "long bean" or "snake bean") has more than 16 ovules and seeds spaced within the pod. Recent molecular evidence suggested that it is a subspecies (Xu et al., 2012; 2010).

Table 5.2. **The five cultivar groups of cultivated cowpea**

Cultivar group	Selected feature
unguiculata	Includes most African grain and forage types. More than 16 ovules/pod.
melanophthalmus	Blackeye pea types. Less than 17 ovules/pod. Grown mostly in the Americas.
biflora (Catiang)	Smooth seed in short erect pods. Common in India. Less than 17 ovules/pod.
sesquipedalis	Asparagus or yard-long beans. Very long pods consumed fresh, especially in the People's Republic of China.
textilis	Rare form with very long peduncles once used for fibre in Africa.

The wild cowpeas in the subspecies *unguiculata* currently are described as being the variety *spontanea* (previously included in the subspecies *dekindtiana*, i.e. in Padulosi [1993]). Var. *spontanea* are similar to domesticated cowpea landraces except that the pods are small and dehiscent, and the seeds are ten times smaller than cultivated cowpea. The seed coat of *spontanea* is hard, thick and impermeable to water. There are no obvious barriers to hybridisation or recombination between members of these five different cultivar groups or with the wild cowpeas (var. *spontanea*) in the subspecies *unguiculata*.

The *Vigna unguiculata* species complex is currently divided into 11 subspecies (Padulosi, 1993; Padulosi and Ng, 1997; Pasquet, 1997, 1993a, 1993b). Ten of the subspecies are perennial and one, cowpea, is annual (Table 5.3). Plants from these subspecies have exhibited varying degrees of crossability with cultivated cowpea. Note that another taxon, *Vigna monantha* Thulin from coastal Somalia, may warrant reclassification as a new *Vigna unguiculata* subspecies.

Table 5.3. **The *Vigna unguiculata* (L.) Walp. subspecies complex**

Subspecies	Perennial	Annual	Habitat
aduensis[2]	Yes		Montane forest areas in Ethiopia north of the Blue Nile (altitude 1 400-2 600 m).
alba[1]	Yes		In the coastal plains from SãoTomé and Gabon to north-western Angola.
baoulensis[2]	Yes		West African rain forest area, from Sierra Leone to eastern Cameroon.
burundiensis[2]	Yes		Mainly found in forest margins, gallery forest margins or cleared grasslands in the subhumid and humid zones in Burundi, Uganda and the Kakamega forest in western Kenya.
dekindtiana[1]	Yes		In semi-arid zones with a disjunct distribution in the mountains from southern Angola and Zimbabwe, and a few specimens observed in northwest Zambia (altitude 1 400-1 900 m) and possibly in West Africa.
letouzeyi[2]	Yes		The Congolese basin rainforest from Cameroon and Gabon to the border of the Democratic Republic of the Congo with Uganda.
pawekiae[2]	Yes		Montane forest of eastern Zimbabwe to south-western Ethiopia through Malawi, eastern Tanzania, Ngorongoro and the major Kenyan mountains. Also observed in the mountains east of Lake Tanganyika (altitude 1 400-2 600 m).
pubescens[1]	Yes		In the coastal Indian Ocean plain from Maputo to Kenya. (A few specimens have also been collected in swamps in Burundi, southern Sudan, south-western Tanzania and Uganda).
stenophylla[1]	Yes		Complex distribution where pubescent forms (var. *protracta* (E. Mey.) Mithen) are in the back of the coastal sand dunes in eastern Cape Province, at higher elevation from Transkei northward, on the eastern slopes of the Drakensberg at 500-1 500 m elevation, in Swaziland and east of Mpumalanga and Northern Province. Narrow leaflet forms (var. *stenophylla* (Harv.) Mithen) occur at low elevations in north-eastern Natal, Swaziland and Kruger Park plain, and at 1 200-1 500 m elevation in the high veld of West Mpumalanga, Gauteng and the northern part of Free State. Scabrous lobed-leaflet forms (var. *kgalagadiensis* Mithen) found in north-eastern Namibia, Botswana, Zambian Barotseland and north-western Zimbabwe.
tenuis[1]	Yes		In two different areas: Zambia-Zimbabwe-Malawi at 1 200-1 800 m and in a coastal area from southern Natal to mid-Mozambique.
unguiculata[1]		Yes	Widely cultivated especially in West Africa (see Figure 5.3).

Notes: 1. Most cultivated cowpeas and the subspecies *alba*, *dekindtiana*, *pubescens*, *stenophylla* and *tenuis* (and var. *spontanea*) are highly self-pollinated. Previously, these subspecies were pooled into the subspecies *dekindtiana* and it is convenient here to call these wild cowpea subspecies the "*dekindtiana* group". 2. The subspecies *aduensis*, *baoulensis*, *burundiensis*, *letouzeyi* and *pawekiae* are all out-crossing. Previously, these subspecies were pooled into the subspecies mensensis and they are described here as the "*mensensis* group".

Description of the plant

The cowpea *Vigna unguiculata* (L.) Walp. is an annual herbaceous legume cultivated for its edible seeds or for fodder. Cultivated cowpeas are herbaceous annuals that are either erect, prostrate or climbing annuals with a tap root and virtually all are glabrous. They are mostly grown for grain but a small proportion (about 10%) are grown as green leafy vegetables and fodder in Africa or as fresh pods in eastern Asia (Boukar et al., 2015).

Cowpea *V. unguiculata* can grow up to 80 cm and up to 2 m for climbing cultivars. It has a well-developed root system. Germination is epigeal with the first pair of true leaves being simple and opposite and subsequent leaves being trifoliate with oval leaflets (6-15 cm long and 4-11 cm broad) and alternate. The papillonaceous flowers are born on racemose inflorescences at the ends of peduncles that arise from leaf axils and can be white, yellowish, pale blue or violet. Peduncles are stout and grooved and usually much longer than the leaves (2-20 cm long). For each inflorescence, flowers are sequentially produced in alternating pairs on thickened nodes at the tip with cushion-like extra-floral nectaries between each pair of flowers. The flower is large (standard is 2-3 cm in diameter), with a straight keel, diadelphous stamens (one free and nine fused), a sessile ovary with many ovules, and a style that is bearded along the inside and ends in an oblique stigma. Pods occur in pairs forming a V, mostly pending and vertical, but they can be erect. They are cylindrical, 2-6 cm long and 3-12 mm broad and contain 8-20 seeds. Seeds can be white, pink brown or black (Heuzé et al, 2013) (Figure 5.1).

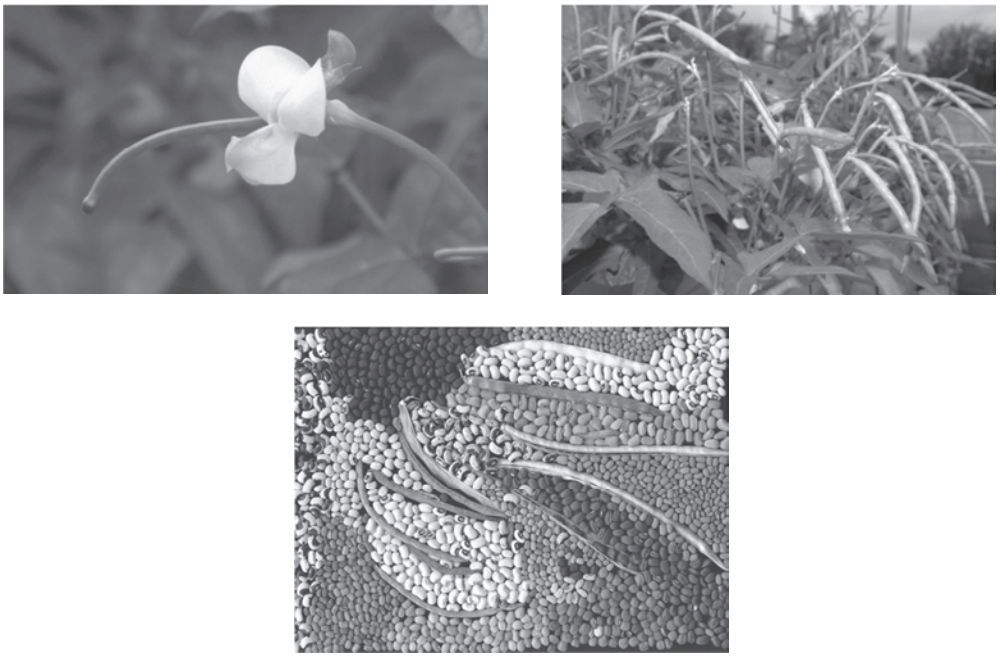
Figure 5.1. **Aerial parts of cowpea (*Vigna unguiculata* (L.) Walp.)**

Note: This line drawing shows leaves, stems, petioles, flowers and pods (main image), together with the reproductive organs consisting of stamens (nine fused and one free) and pistil with its curved style with brush below the stigma (bottom left) and parts of the corolla (bottom right); the standard (top), two wings (middle) and keel (bottom).

Source: Steward (1958), digitized by BHL wiki and licensed under CC BY-NY-SA 4.0.

The corolla is yellowish-white to violetish-white with violet wings and mature seed colours vary from white through brown to black (Figure 5.2).

Figure 5.2. **Cultivated cowpea flower, pods and seeds**



Note: Picture of cowpea flower (top left), immature green pod (top right), maturing pods with an illustration of the great variety of seed colours (bottom).

Source: Courtesy Carl Davies, CSIRO.

Cultivated cowpeas are mostly indeterminate and some have the potential to produce multiple flushes of flowers (Gwathmey, Hall and Madore, 1992) that live for less than one year. The wild relatives of cowpeas, which are perennial (Table 5.3), have fleshy roots and the capacity to resprout after a dry or cool season.

Geographic distribution, habitats, crop production, centres of origin and diversity

Geographic distribution

Cultivated cowpeas are grown as warm-season-adapted annuals in tropical and subtropical zones (as defined by Hall [2001]) in all countries in sub-Saharan Africa and in Asia, South America, Central America, the Caribbean, the United States and around the Mediterranean Sea. In subtropical zones temperatures are only suitable for cowpea in the summer, whereas temperatures are suitable year-round in tropical zones. The vast majority of the world's cowpea production (over 95%) takes place in sub-Saharan Africa (Figure 5.3), with about 12.5 million hectares under cultivation worldwide in 2014 (Singh et al., 2002; FAOSTAT, 2014) (Table 5.4). Asia is the second largest producing region, representing less than 3% of the global production in average over the 1993-2014 period (Figure 5.3), most of it being cropped in Myanmar (FAOSTAT, 2014).

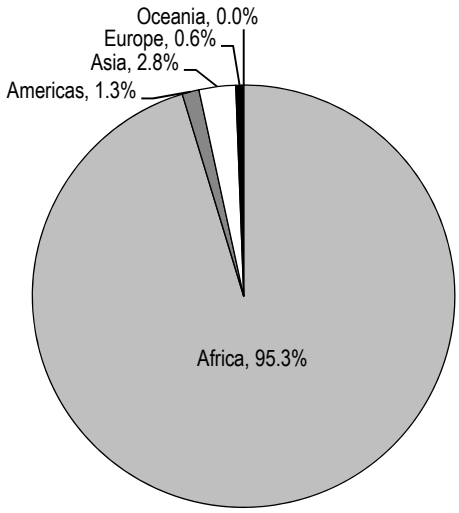
In Africa, cowpea can be cultivated up to 1 800 m altitude but is mainly grown in the lowlands. The centre of maximum diversity of cultivated cowpeas and land races is found in West Africa in a region comprising the Sudan savannah zone of Nigeria (at 4 million ha, Nigeria has the largest area of cowpea cultivation according to FAOSTAT),

Table 5.4. **Global production of cowpeas (dry) in million metric tonnes (MMT)**

Cowpea production	Average 1993-2014	2010	2011	2012	2013	2014
World	**4.59**	**6.91**	**4.78**	**8.25**	**8.03**	**5.59**
Africa	4.37	6.57	4.50	7.95	7.78	5.35
including – Nigeria	*2.53*	*3.37*	*1.64*	*5.15*	*4.63*	*2.14*
– Niger	*0.79*	*1.77*	*1.52*	*1.33*	*1.63*	*1.59*
– Burkina Faso	*0.37*	*0.63*	*0.44*	*0.60*	*0.58*	*0.57*
– Tanzania	*0.13*	*0.15*	*0.17*	*0.18*	*0.19*	*0.19*
– Cameroon	*0.10*	*0.15*	*0.16*	*0.15*	*0.17*	*0.17*
– Mali	*0.10*	*0.13*	*0.13*	*0.14*	*0.17*	*0.15*
– Kenya	*0.07*	*0.07*	*0.08*	*0.11*	*0.13*	*0.14*
Asia	0.13	0.23	0.19	0.19	0.15	0.15
including – Myanmar	*0.11*	*0.21*	*0.17*	*0.16*	*0.12*	*0.12*
Americas	0.06	0.09	0.07	0.09	0.08	0.07
Europe	0.03	0.03	0.03	0.02	0.02	0.02
Oceania	0.00	0.00	0.00	0.00	0.00	0.00

Source: FAOSTAT (2014).

Figure 5.3. **Cowpea production share by region, average, 1993-2014**



Source: FAOSTAT (2014).

central Burkina Faso, Ghana, Togo, northern Benin and the north-western part of Cameroon (Padulosi and Ng, 1997). Substantial cowpea cultivation also occurs in the semi-arid Sahelian zone, which is a transition zone between the Sahara desert in the north and the Sudan savannah zone in the south. The Sahel encompasses northern and central Senegal and southern Mauritania in the west to central Sudan in the east, passing through central Mali, northern Burkina Faso, southern Niger (at 5 million ha, Niger has the largest area of cowpea cultivation) and central Chad. Significant cowpea production also occurs in the northern Guinea savannah zone and the forest and southern Guinea savannah zones of West Africa, the United Republic of Tanzania and Uganda, and some cowpeas are cultivated in central, southern and north-eastern Africa. Many areas where cultivated

cowpeas are grown and the locations where the wild cowpea *V. unguiculata* var. *spontanea* has been found are shown in Figure 5.4.

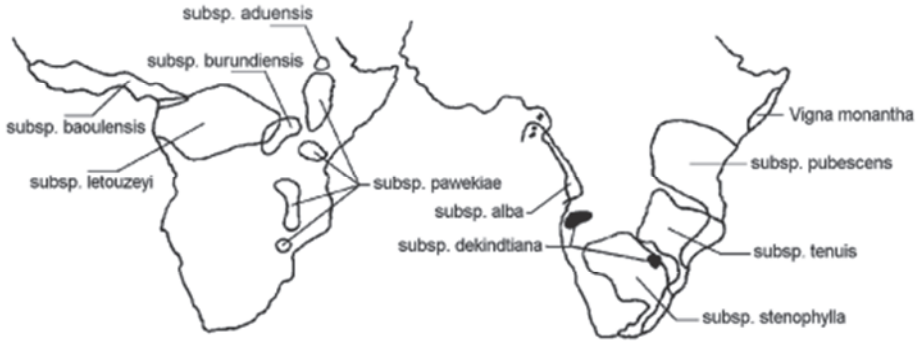
Figure 5.4. **Distribution of cultivated and wild cowpeas in Africa**

Note: Areas with cultivated cowpea are shown in grey, while the black dots indicate the locations where wild cowpea *V. unguiculata* var. *spontanea* occurs.

Source: Adapted from Remy Pasquet.

The wild relatives of cowpea are widely distributed across sub-Saharan Africa (Figure 5.5). They occupy a range of habitats (described in Table 5.3) to an elevation of 2 600 m. *Vigna monantha* has been found in Somalia in the coastal plain from Hobyo to Bender Bayla.

Figure 5.5. **Distribution of the wild relatives of cowpea in Africa**



Source: Adapted from Pasquet (1996).

In Asia, cowpea ("asparagus bean") ranks as one of the top ten fresh vegetables. It is cultivated across a broad geographic range, except for some permanently cold regions. According to the FAO statistics, Myanmar is the main cowpea producer in Asia (FAOSTAT, 2014). China, India, Japan, Korea and Thailand are among the major asparagus bean-producing countries. The estimated annual cultivation area in Asia in total

is 1 million ha, China alone making up roughly one-fifth of the world's fresh pods production with over 1.5 million tonnes (equivalent to an additional 0.2 MMT of dry matter). Compared with the African cowpea, "asparagus bean" is more adapted to cool climates and is less tolerant to very high temperatures.

Ecosystems and habitats of native and naturalised cowpea

Cowpeas and their wild relatives have persisted for thousands of years in sub-Saharan Africa with many occurring in West Africa and southern Africa. While some wild relatives are persistent from year to year due to their fleshy roots and ability to resprout after a dry or cool season, most wild relatives persist through the production of hard seed that can remain viable for several years in the soil.

The wild cowpeas *V. unguiculata* var. *spontanea* clearly benefit from human disturbance as shown in the following examples from the Africa region. In the Milalani wild population in coastal Kenya, the population has increased after each mechanical clearing of the roadsides. In a long-term seed-supplementation trial in Muhaka field station in Kenya, the plots that were ploughed every year had more wild cowpea plants than the undisturbed plots (R.S. Pasquet, personal communication). While *Vigna unguiculata* var. *spontanea* can be found in natural ecosystems from Cameroon eastward with clear examples in eastern Cameroon, Uganda and the western Ethiopian lowlands, it seems only to be found in disturbed places (fields, field margins, roadsides and fallows) in Burkina Faso, western Niger and northern Ghana. In the West African Sahel, cowpea is also widely cultivated for fodder. For farmers mainly focusing on fodder, fodder from wild cowpea (as well as domesticated-wild F_1 hybrids and their progenies) may be considered as being equivalent to fodder from domesticated cowpea. Often wild cowpea plants are not uprooted from the field, and appear to be tolerated in the agro ecosystem. The hybrid progenies may even end up being used by farmers for sowing and may be considered as fodder landraces. Wild cowpeas and wild relatives of cowpea do not appear to represent a significant weed problem in sub-Saharan Africa (Huesing et al., 2011).

Those few cowpea landraces that produce some hard seeds that can survive for several years in the soil may have a tendency to persist in and around cultivated fields. Domesticated cowpea can theoretically survive as feral plants, as was shown for example in Japan (Berville et al., 2005). However, this rarely has been observed in Africa; for example, a few small feral populations observed in coastal Kenya were not seen in consecutive years.

Centres of origin and diversity

Several hypotheses have been proposed for the domestication of cowpea in different parts of sub-Saharan Africa (summarised in Ba, Pasquet and Gepts, 2004). It is likely that cowpea was domesticated only once, probably in West Africa about 2000 B.C. (Padulosi and Ng, 1997), and that the progenitor of cultivated cowpea was the wild cowpea *V. unguiculata* var. *spontanae* (Pasquet, 1999). In West Africa, where most of the world's cowpea is cultivated, there are many weedy forms that are intermediates between truly wild forms and very small-seeded cultivated cowpeas (Rawal, 1975). Recent molecular evidence shows that the "asparagus bean" has undergone a severe genetic bottleneck during domestication in Asia from its African progenitors (Fang et al., 2007; Xu et al., 2010).

The greatest genetic diversity in wild relatives of cowpea has been found in southern Africa in a region encompassing Namibia from the west, across Botswana, Zambia,

Zimbabwe and Mozambique to the east, and South Africa and Swaziland to the south (Padulosi and Ng, 1977). This genetic diversity includes many primitive traits that were lost in domestication such as perenniality, hairiness, small size of seeds and pods, hard seeds, pod shattering and outbreeding. Cultivated cowpeas also are present in this region. The South African Transvaal may have been the centre of speciation of *Vigna unguiculata* due to the presence there of the most primitive subspecies (Padulosi and Ng, 1977).

Crop production and management practices

Africa

Most cowpea grown in the African region is intercropped with sorghum (*Sorghum bicolor*) or pearl millet (*Pennisetum glaucum*), and sometimes with other crops such as maize (*Zea mays*), cassava (*Manihot esculenta*) or cotton (*Gossypium* spp.) (Blade et al., 1996). The crop is typically planted at wide spacing (1 m) irregularly through young stands of the component cereal or other crop. Because the cowpea is planted after cereal crop establishment, at low density and without inputs, dry grain cowpea yields in the range of 300 kg/ha only are typically achieved in such systems. In Senegal, most of the cowpea production is sole-cropped (Thiaw, Hall and Parker, 1993), in part due to the light sandy soils and availability of horse-drawn peanut seed drill which can easily be modified to plant cowpea in rows, making possible animal-draft cultivation to control weeds. In the last decade, an increasing portion of the cowpea crop in other parts of Africa has been planted in pure stand, at relatively higher density, using improved varieties and with agricultural inputs, especially insecticides, resulting in average yields of between 1-2 tonnes/ha. Strong demand for cowpea-based foods in urban areas and good prices are driving this transition to more intensified production practices.

Figure 5.6. **Cowpea field, Shawula district, Swaziland**

Source: Courtesy EcoPort (www.ecoport.org). Author Roger P. Ellis.

Cowpea is a legume species usually considered as being resistant to droughts. Droughts often occur in the Sahelian zone and Sudan savannah zones (Dancette and Hall, 1979). Cowpea has a greater ability to withstand these droughts and to produce significant grain than any other crop grown, including the drought-resistant crops pearl

millet, sorghum and peanut. In addition, cowpea hay is an important source of forage for livestock, which plays a particularly critical role in feeding animals during the dry season in many parts of West Africa (Singh and Tarawali, 1997; Tarawali et al., 2002, 1997).

Figure 5.7. **Cowpea (*Vigna unguiculata* (L.) Walp.) straw as feed for cattle**

Source: IITA Image Library, licenced under CC BY 3.0.

Other regions of the world

In Asia and Brazil, both sole-cropping and intercropping are practiced (Pandey and Ngarm, 1985; Watt, Kueneman, and de Araújo, 1985), while in the United States generally only sole-crops are grown. In Brazil and India, some intercropping of cowpea is still practiced, but the majority of the crop is produced under sole-cropping with inputs. Cowpea production in the United States is entirely mechanised with machinery and agronomic practices adapted from other crops such as common beans or soybeans. Large growers in Brazil have adopted similar modern farming practices to produce high yields (Freire Filho et al., 2011).

In China, "asparagus bean", as a vegetable, is usually intercropped with common bean or cucumber. Smallholder farming and hand-harvest of the immature fresh pods of asparagus bean still remains the dominant production system in China, as pod quality/appearance, rather than yield, is usually more important.

Reproductive biology

Generation time and cropping season duration

Domestic cowpeas

Domesticated cowpeas are annuals with duration from sowing to harvest varying from two to six months. Cowpeas are grown as a rainfed crop and the dates of sowing and maturity must fit the timing of the rainfall and the hydrologic budget (Dancette and Hall, 1979). Cultivars vary in their responses to photoperiod and temperature as they influence the time of budding and flowering. A classification of these responses by Ehlers and Hall (1996) includes three photoperiod classes (day-neutral, quantitative short-day and obligate short-day), three juvenility classes (short, intermediate and long), three classes of

heat-induced floral bud suppression (no bud suppression, partial and complete bud suppression) and two classes of pod-setting ability under hot long days (low and high). Semi-arid, subhumid and humid zones are considered as they were defined by Hall (2001).

In the semi-arid Sahelian zone of Africa, where the growing season usually is very short due to a short rainy season, adapted cowpea cultivars include:

- erect day-neutral ones with a short juvenile period that have a cycle length of 60 days
- spreading day-neutral ones with a slightly longer juvenile period that have a cycle length of 70 days (Hall, 2004)
- dual-purpose, spreading, short-day ones with a longer cycle of about 90 days for producing hay and grain.

Note that day-neutral cultivars have a fairly constant cycle length because time of flowering is not influenced by photoperiod, but is rather influenced by temperature which is relatively constant in tropical zones.

In the wetter semi-arid Sahelian and subhumid Sudan savannah zones to the south, adapted cowpea cultivars include ones with different types of short-day requirements for flowering. The beginning of the rainy season, which determines the time of sowing, can be much more variable than the end of the rainy season, which determines the optimum time for harvest. Adapted cowpea cultivars with an appropriate short-day requirement reach maturity at the optimum time for harvest even with substantial variation in sowing date. Thus, these cultivars have a variable cycle length depending on the date of sowing.

Further south in the wetter subhumid Sudan and humid Guinea savannah zones, cowpea cultivars may be found that are day-neutral but have a long cycle length due to a long juvenile period (Lush, Evans and Wien, 1980).

Most Chinese "asparagus bean" cultivars are day-neutral or weakly short-day.

Wild relatives of cowpea

With respect to the wild relatives of cowpea, members of the *dekindtiana* group that are adapted to the Sudan savannah zone were observed to be obligate short-day plants (Lush, Evans and Wien, 1980). Members of *V. unguiculata* var. *spontanae* also are short-day plants. In contrast, members of the mensensis group, which are adapted to the more humid forest and southern Guinea savannah zones, were observed to be day-neutral with a long juvenile period (Lush, Evans and Wien, 1980). In areas of East Africa where there is a bimodal rainy season, wild relatives of cowpea have been observed to have a cycle length of one to two years. They germinate during the beginning of one rainy season and produce fruits during this rainy season, and then survive the dry season using carbohydrate reserves in the fleshy roots and grow again at the commencement of the next rainy season producing more fruits and then survive the dry season. These wild relatives of cowpea are presumed to be day-neutral in their flowering behaviour.

Reproduction characteristics

Pollen dispersion

There is no mechanical dispersion of pollen from the flowers of cultivated cowpeas because the anthers release pollen during the first half of the night when the flowers are

still closed (Ladeinde and Bliss, 1977), and the pollen is sticky and heavy. The cuticle which protects the stigmatic surface breaks and releases a stigmatic exudate during the second half of the night at which time self-fertilisation can begin. Subsequently, the flower opens during the early morning and then closes in the late morning.

Pollination characteristics

In general, cultivated cowpeas have a high level of self-pollination. Based on their work in Texas, Blackhurst and Miller (1980) noted that the pollination process in cultivated cowpeas is complete before the flower opens. However, once they have begun flowering, cultivated cowpeas, wild cowpeas and wild relatives have the ability to produce flowers every day for several weeks (Gwathmey, Hall and Madore, 1992). Consequently, some opportunities for cross-pollination occur providing pollinators are present. Outcrossing in limited amount has been observed and quantified in literature. Fatokun and Ng (2007) report it at two locations in Nigeria and one location in Benin, and in one case pollen travelled up to 31 m between parental plants. The authors concluded that outcrossing occurred at a frequency of less than 1%. In Senegal, outcrossing rates at 2% have been observed. In the south-eastern United States, outcrossing of 0-1.4% was observed with six cultivars (Williams and Chambliss, 1980). Some non-quantitative observations have also been made. Significant outcrossing has been observed in cowpea fields that are next to wild lands in Botswana. In California, some cowpea cultivars have exhibited a few percent outcrossing in some locations.

Cross-pollination is usually less than 1%, but will vary somewhat with the cultivar and, more particularly, with the population of some insects. In several cases, the pollinators are not known, but honeybees (*Apis mellifera*) have been observed around cowpea flowers and thus have been implicated in pollination (Ige, Olotuah and Akerele, 2011). Purseglove (1968) reported that the extra-floral nectaries at the base of the corolla attract ants, flies and bees, but noted that a heavy insect would be required to depress the wings of the flower and expose the stamens and stigma (tripping). In coastal Kenya and Burkina Faso, several large carpenter bee species (*Xylocopa* spp.) and leafcutter bee species (*Megachilidae* spp.) were considered potential cross-pollinators of cowpea (R.S. Pasquet, personal communication), and it was shown that these same leafcutter and carpenter bees were the likely pollinators of the wild progenitor of cowpea (Kouam et al., 2012). Casual observations made in California and Texas (United States) and Nigeria indicate that large bumblebees (*Bombus* spp.) may be responsible for the cross-pollination that occurs in cowpeas in these regions.

Inter-specific crossing between wild and cultivated cowpeas are rare (see the description under the section "Species/subspecies hybridisation and introgression" on the next page).

Seed viability

Cultivars of domesticated cowpeas usually do not create long-lived seed banks in the soil because their seed coats typically are permeable to water and the seeds have little dormancy (Lush, Evans and Wien, 1980). Some land races and cultivars with smooth seed coats can have some hard seeds.

Wild cowpeas and relatives of cowpea have dormant seeds due to the impermeable nature of their seed coats (Lush and Evans, 1980). These hard seeds can survive for several years in the soil, especially if the soil is dry.

Genetics and genome mapping

Cowpea is a diploid with 2n = 2x = 22 chromosomes, one of which is short (19 µm), 7 are medium length (26-36 µm) and 3 are long (41-45 µm) (Frahm-Leliveld 1965; Mukherjee 1968). The genome size is about 613 Mb (Arumuganathan and Earle, 1991). Chloroplasts are maternally inherited (Corriveau and Coleman, 1988). The wild subspecies also are diploid with 2n = 22 (Vikal and Satija, 1992; Venora and Padulosi, 1997; Adetula, 2006).

Much progress has been made recently in developing genetic maps of cowpea using a range of methods: restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), genomic scar markers (SCAR), simple sequence repeat (SSR), single-nucleotide polymorphism (SNP) and phenotypic markers (Timko, Ehlers and Roberts, 2007; Andargie et al., 2011; Lucas et al., 2011) together with information on genome organisation (see the Cowpea Genomic Initiative developed by the Department of Biology of the University of Virginia at: *http://cowpeagenomics.med.virginia.edu*).

Of note is the recent construction of a high-density cowpea consensus genetic map based on SNP markers together with information on genome organisation (Muchero et al., 2009; Lucas et al., 2011). An SNP-based genetic map has also been constructed for asparagus bean (Xu et al., 2011).

Based on these platforms, quantitative trait loci (QTLs) governing many agricultural and adaptive traits such as leaf morphology, foliar thrips resistance and drought tolerance, have been mapped (Muchero, Ehlers and Roberts, 2010a, 2010b; Muchero et al., 2009; Pottorff et al., 2012). A high quality bacterial artificial chromosome- (BAC-) based physical map is also available for cowpea (790 contigs and 2 535 singletons), and the genome assembly of cowpea is underway (Close et al., 2011).

Species/subspecies hybridisation and introgression

Natural interspecific crossing (extent, sterility/fertility)

Floral morphology favours either autogamy (self-pollination) or allogamy (outcrossing) in different groups of the *V. unguiculata* species complex. Most cultivated cowpeas and members of the *dekindtiana* group are highly self-pollinating in that their anthers usually are in contact with their stigmatic surface. The mensensis group of subspecies exhibits high levels of outcrossing and has anthers that are a few millimetres below the stigmatic surface, with the stigmatic surface oriented upwards and its lower part protected by a beard of long hairs (Lush, 1979).

To date, no successful natural or artificial crosses have been reported and subsequently confirmed between any member of the *Vigna unguiculata* species complex and any other species. Although *Vigna schlechteri* and *Vigna vexillata* are the closest species to *Vigna unguiculata*, numerous attempts to cross either of these species with *V. unguiculata* have failed (Mithen, 1989; Barone, Del Giudice and Ng, 1992; Fatokun, 2002; Fatokun, Perrino and Ng, 1997).

Wild cowpeas in the mensensis group with floral morphologies that favour outcrossing function differently than the cultivated cowpea. If their flowers are not tripped by a heavy bee, they may remain open until late into the afternoon (Lush, 1979) and can eventually reopen the following morning. This wild cowpea group has much higher levels of cross-pollination than cultivated cowpeas, but does not readily cross with

cultivated cowpea. Studies have been conducted in coastal Kenya with cultivated cowpea and a wild cowpea *V. unguiculata* var. *spontanea* that had an outcrossing floral morphology. The level of outcrossing was less than 2%. Cultivated cowpeas readily cross with wild cowpeas in the same subspecies (i.e. var. *spontanea*) and can be crossed with members of the other subspecies of *Vigna unguiculata* but with varying degrees of difficulty.

Experimental crosses

The subspecies from the mensensis group are not readily crossed with cultivated cowpea although it is possible, while some subspecies from the *dekindtiana* group are more easily crossed with cultivated cowpea (Sakupwanya, Mithen and Matangandura-Mhlanga, 1989; Kouadio et al., 2007, 2006). Breeders working with the subspecies *dekindtiana* have obtained many viable progeny after a simple hybridisation with cultivated cowpeas. In contrast, with plants from the subspecies *pubescens*, they have found it useful to backcross the F_1 with a parent because most of the F_1 seed were shrivelled and had low levels of germination and emergence. Crossability of plants from the subspecies *tenuis* with cultivated cowpeas has been found to be intermediate in ease between *dekindtiana* and *pubescens*.

The overall message is that crosses appear possible among all members of the *Vigna unguiculata* complex but they vary from being easy to being difficult.

Information and data on introgression

A very high frequency of progeny from naturally formed interspecific hybrids between wild and cultivated cowpeas would have one or more domestication traits that significantly reduce their persistence in wild ecosystems. However, as feral wild x cultivated plants are sometimes used for forage by farmers, it is likely that hybridisation between such plants and wild cowpeas will occur and that the progeny would have an essentially wild phenotype with high survival potential in natural ecosystems.

General interactions with other organisms (ecology)

Potential positive effect of cowpea on cereal production

Cultivated cowpeas play a critical role in the cereal-based intercropped and rotational cropping systems where they are often grown in sub-Saharan Africa, in terms of nutrient improvement and resistance to certain pests.

Cultivated cowpeas have symbiotic relations with rhizobia (Elowad and Hall, 1987) and mycorrhizae (Kwapata and Hall, 1985) that enhance the flow of reduced nitrogen and phosphate into the cropping system. These nutrients frequently limit the productivity of cereals in sub-Saharan Africa, and associated legumes can bring a beneficial effect.

Certain cowpea genotypes can cause suicidal germination of the seeds of the weed parasite *Striga hermonthica*, which is a major pest of pearl millet, sorghum and maize that has been difficult to solve by other means (Singh and Matsui, 2002). Some cowpea genotypes can reduce the reproduction of certain plant parasitic nematodes (including *Scutellonema cavenssi*) that can damage pearl millet, sorghum and peanut (Germani, Baujard and Luc, 1984; Hall et al., 2003).

Consequently, cowpea can enhance the edaphic conditions and thus the productivity of the cereals and other crops that are grown in rotation or as intercrops with it. An increase in the area of cowpea cultivation over present levels in sub-Saharan Africa

would not only benefit cereal productivity but also livestock production, whole farming systems and human nutrition and welfare.

Pests and diseases

Cowpeas are host to a range of pests and diseases such as insects and mites, viruses, fungal and bacterial diseases, nematodes and parasitic weeds. These may affect the whole plant, the flower or the pod and are detailed in Annex 5.A1, together with information on plant resistance and methods for pest control and management. The pests of major economic importance are *Maruca vitrata*, *Aphis craccivora*, *Clavigralla tomentosicollis*, *Megalurothrips sjostedti* and *Callosobruchus maculatus*.

Human health and biosafety

Like other grain legumes, cowpeas contain a range of anti-nutritional factors such as hemagglutinin, tannin, trypsin inhibitors, oxalate, phytate, polyphenols and oligosaccharides (Sreerama et al., 2012; Afiukwa et al., 2012). The levels of anti-nutritional factors in cowpea are similar to those in the widely consumed food legume, chickpea (see Table 5.5).

Table 5.5. **Anti-nutritional factors in the grain of chickpea and cowpea**

Anti-nutritional factor	Chickpea	Cowpea
Phytic acid (mg/g)	12.1	14.0
Polyphenols (mg GA/g)	10.8	12.1
Oligosaccharides (mg/g)	34.9	31.7
Raffinose	8.6	10.3
Stachyose	19.1	17.8
Verbascose	7.2	3.6
Trypsin inhibitor activity (Units/g)	6 452	6 981
Trypsin inhibitor activity [IC_{50} (μg/ml)]	44.8	38.2

Source: Adapted from Sreerama et al. (2012).

Cowpea grains complement the grains of cereals as foods for people by enhancing the quantities and qualities of proteins and vitamins. For example, cowpea grains have substantial levels of folic acid, which is a critical vitamin for all people and especially pregnant women since it prevents the occurrence of neural tube defects such as spina bifida in infants. Fresh and dry grains of early season cowpea cultivars and fresh pods and leaves are often an important source of food during the "hungry period" occurring two months prior to the main cereal harvest in the Sahelian and savannah zones (Dancette and Hall, 1979). Cowpea is a staple crop having a greater ability to withstand these droughts and to produce significant grain than any other agricultural plant grown in these zones, including the drought-resistant grain crops pearl millet, sorghum and peanut (Turk, Hall and Asbell, 1980; Ziska and Hall, 1983; Petrie and Hall, 1992; Singh and Matsui, 2002; Hall, 2004).

The grain is the most important part of the cowpea plant for human consumption. The seeds are most often harvested and dried for storage and consumption at a later time, either after cooking whole or after being milled like a flour product and used in various recipes (Nielsen, Ohler and C. Mitchell, 1997; Ahenkora, Adu Dapaah and Agyemang, 1998). As such, cowpea plays a critical role in the lives of millions of people in the developing world, providing them a major source of dietary protein that nutritionally

complements low-protein cereal and tuber crop staples. The nutritional profile of cowpea grain is similar to that of other pulses, with a relatively low fat content and a total protein content that is two- to fourfold higher than cereal and tuber crops. Similar to other pulses, the storage proteins in cowpea seeds are rich in the amino acids lysine and tryptophan when compared to cereal grains, but low in methionine and cysteine when compared to animal proteins. Total seed protein content ranges from 23% to 32% of seed weight (Nielsen, Brandt and Singh, 1993; Hall et al., 2003; Boukar et al., 2011).

In the south-eastern parts of the United States, portions of West Africa, Asia, and in the Caribbean, consuming fresh seeds and green pods is preferred to the cooked dry seeds (Nielsen, Ohler and C. Mitchell, 1997; Ahenkora, Adu Dapaah and Agyemang, 1998). In many parts of Africa and Asia, in addition to the seeds, the fresh or dried leaves are also consumed as a side dish or as part of a stew and provide significant nutritional value. In addition to human consumption, cowpea leaves and stems (stover) are also an important source of high-quality hay for livestock feed (Tarawali et al., 2002; 1997). Fresh pods of asparagus bean provide people in Asia with a source of energy protein, multiple vitamins and minerals.

Annex 5.A1. Common pests and pathogens

Cowpea pests and economic consequences

There are many pests and diseases of cowpea (Table 5.A1.1) although insects tend to be the most economically important. There are good levels of host plant resistance for many of these pests in the cowpea germplasm, and it is being successfully deployed by the cowpea breeders.

However, there are several important pests for which strong cultivar resistance is not available in the primary gene pool. These are flower thrips (*Megalurothrips sjostedti*), pod-sucking bugs (*Clavigralla tomentosicollis*) and the podborer (*Maruca vitrata)* (Jackai and Daoust, 1986; Jackai and Adalla, 1997; Dreyer, Baumgärtner and Tamò, 1994). About two to three sprays of insecticide are needed to prevent significant economic losses by: 1) flower thrips reducing flower production; 2) pod-sucking bugs reducing pod and seed development; and 3) podborers damaging peduncles, floral buds, flowers, green pods and developing grain. Most African farmers do not apply insecticides to cowpea and as a consequence grain yields are 10-20% of what might be obtained with a complete spraying regimen (Jackai and Adalla, 1997).

Cultivated cowpea flowers are also visited by forage bees. In Africa, several bees have been observed on cowpea flowers (Table 5.A1.3) (Pasquet et al., 2008; Asiwe, 2009; Ige, Olotuah and Akerele, 2011).

Podborer

Many scientists consider the podborer to be the most damaging and economically important insect pest of cowpea in sub-Saharan Africa except for in the Sahelian zone, where it rarely occurs. In reviewing the biology of the podborer, Singh and Jackai (1985) noted that the female moth lays up to 200 eggs on flower buds, flowers and tender leaves of cowpea. Eggs hatch in two to three days, and there are five larval instars. Larval development takes about 8-14 days. The late larval instars can be identified by the black dots on their body. A two-day prepupal period follows the larval period, during which feeding ceases. The pupal stage takes six to nine days, and the pupae are initially green or pale yellow but later darken to greyish brown. Pupation occurs in the soil in a double-walled pupal cell, and adults emerge after about 5-10 days and have a life span of 5-15 days. The early larvae, in the absence of flower buds and flowers, feed on young tender shoots and peduncles. Later, when the flower buds and flowers are formed, they move to and feed on floral parts and subsequently on green pods. Pod damage consists of tunnelling by foraging larvae and is particularly dramatic, hence the common name of this insect. Infested pods are often webbed together with leaves, flowers and other pods.

The International Institute of Tropical Agriculture (IITA), headquartered in Nigeria, has devoted much effort over three decades to developing methods for controlling podborer in cowpea (Oghiakhe, Jackai and Makuanjuola, 1995; Jackai, Padulosi and Ng, 1996). At this time there is no domesticated cowpea with adequately strong resistance to podborer (Adekola and Oluleye 2008), and conventional breeding may have little chance of producing cowpea cultivars with adequate resistance to podborer (Machuka, 2002).

Resistance to stem damage is available in many cultivars, but high levels of resistance to feeding damage in flowers and pods is not available in cultivated cowpeas (Jackai, Padulosi and Ng, 1996). There is some evidence that pods held together at a wide angle above the crop canopy suffer less damage than pods produced within the canopy and separated by a narrow angle (Oghiakhe, Jackai and Makuanjuola, 1995; Singh, 1980). Cultivars with pods held above the canopy are useful but have a disadvantage. Pods are not very active in photosynthesis and when above the canopy, they reduce the amount of solar radiation reaching the leaves. Studies with cowpea genotypes having different canopy architecture indicated the pods-above-the-canopy trait can reduce photosynthetic efficiency and crop growth rates by as much as 54% (Kwapata, Hall and Madore, 1990). Variations in crop management practices such as cowpea spacing (Asiwe et al., 2005) or sole cropping versus various types of intercropping (Jackai and Adalla, 1997) were shown to have little influence on the populations of podborer or the damage they cause to cowpea.

The use of plant-derived insecticides to control podborer has been studied with emphasis on the neem tree (*Azadirachta indica* A. Juss). Extracts from the kernel, seed and leaves of neem have been shown to cause growth disruption, feeding inhibition, deterrence and mortality in podborer but they are not as effective as synthetic insecticides (Jackai and Adalla, 1997). Applying pesticidal forms of *Bacillus thuringiensis* to control podborer has had limited success (Taylor, 1968). This pesticide is broken down by the ultraviolet rays of the sun and usually is only effective for a few hours.

Attempts to develop biological control methods for podborer have failed in the past (Waterhouse and Norris, 1987). More recent research suggests that the podborer is native to southeastern Asia and its parasitoids are being sought in south-east Asia and tested for their efficacy and specificity (Tamò et al., 1997). Currently, biological control methods are being actively studied and several promising candidates (Table 5.A1.2) are emerging (Tamò et al., 2012).

Use of synthetic insecticides is considered the most effective and dependable means for controlling podborer in cowpea (Asiwe et al., 2005). Insecticides are often not locally available or are too expensive for smallholder farmers. Health problems related to misuse of insecticides (Coulibaly and Lowenberg-DeBoer, 2002; Maumbe and Swinton, 2003) are another reason for considering alternative solutions to the podborer problem.

Hairy caterpillar

In the Sahelian zone, which is the second most important area where cowpeas are grown, insect pest pressure is low but on occasions hairy caterpillar (*Amsacta moorie* Butler syn. *Amsacta moloneyi* Druce) can totally destroy large areas of the crop and cultivar resistance is not available. At the beginning of the rainy season in the Sahelian zone of Senegal, waves of female *Amsacta* moths emerge and lay eggs on a large range of plant species (Ndoye, 1978). They will feed on a range of grasses, pearl millet, sorghum and peanut but they show preference for cowpea. If the cowpea plants are young when they are infested, they are defoliated and killed. If the cowpea plants are large, they can outgrow the attack and are only partially defoliated. Usually, however, the waves of hairy caterpillars arrive when the cowpea plants are young.

Hairy caterpillar can be controlled by synthetic insecticides; however, farmers usually do not have the spraying equipment or supplies of insecticide to enable them to control the sporadic large waves of hairy caterpillar that occasionally occur in the Sahelian zone.

In cases where hairy caterpillar is not present, useful yields of cowpea often can be obtained in the Sahelian zone without using insecticides, which is one reason why many farmers in this zone do not have either sprayers or insecticides.

Table 5.A1.1. **Pests and diseases of cowpea**

Insects and mites	Podborers – *Maruca vitrata** – *Cydia ptychora* Hairy caterpillar (*Amsacta moorie*)* Storage pests – *Callosobruchus maculatus** – *Bruchidius atrolineatus* Thrips – *Megalurothrips sjostedti** – *Sericothrips occipitalis* – *Frankliniella schultzei* Pod-sucking bugs – *Clavigralla tomentosicollis** – *Riptortus dentipes* – *Anoplocnemis curvipes* Lygus bugs (*Lygus hesperus*) Cowpea curculio (*Chalcodermus aeneas*) Stink bugs (*Nezara viridula*) Aphids – *Aphis craccivora* – *Myzus persica* – *Aphis gossipii* Green leafhopper (*Empoasca kraemeri*) Foliage beetles – *Ootheca mutabilis* – *Medythia quaterna* Flower beetle (*Mylabris pustulata*) Greasy cutworm (*Agrotis psilon*) Bean shoot fly (*Ophiomyia phaseoli*) Bean pod fly (*Melanogromyza sojae*) Red spider mite (*Tetranychus urticae*)	Fungal and bacterial diseases	Septoria leaf spots – *Septoria vignae* – *S. vignicola* Scab (*Elsinoë phaseoli*) Brown blotch (*Colletotrichum capsici* and *C. truncatum*) Cercospora leaf spot (*Cercospora canescens*) Fusarium wilt (*Fusarium sp*) Rusts – *Uromyces appendiculatus* – *Phakopsora pachyrhizi* Anthracnose (*Colletotrichum destructivum*) Powdery mildew (*Erysiphe polygoni*) Ashy stem blight (*Macrophomina phaseolina*) Ascochyta blight (*Ascochyta phaseolorum*) Pythium stem rot (*Pythium aphanidermatum*) Sclerotium stem rot (*Sclerotium rolfsii*) Bacterial blight (*Xanthomonas campestris*) Bacterial pustule (*Xanthomonas axonopodis*)
Viruses	Cowpea aphid-borne mosaic virus (CABMV)** Blackeye cowpea mosaic virus (BICMV)** Cucumber mosaic virus (CMV)** Cowpea mosaic virus (CPMV)** Cowpea severe mosaic virus (CSMV)** Southern bean mosaic virus (SBMV)** Cowpea mottle virus (CPMoV)** Cowpea golden mosaic virus (CGMV) Cowpea chlorotic mottle virus (CCMV)	Nematodes	Root knot nematode – *Meloidogyne incognito* – *M. javanica*) Cyst nematode (*Heterodera* spp)
Parasitic weeds	Striga (*Striga gesnerioides*) Alectra (*Alectra vogelii*)		

Notes: * No strong host resistance. ** Seed-borne viruses.

Pest predators

As in all cropping systems there are a variety of natural enemies feeding/developing on cowpea insect pests. These natural enemies include more than 25 parasitoid species belonging to the families listed in Table 5.A1.2 (Jackai and Daoust, 1986; Bottenberg, Tamò and Singh, 1998; Adati et al., 2008). In addition to parasitoids, generalist predators

also feed on cowpea insect pests (Table 5.A1.3). These include mites, beetles, ants, bugs and spiders (Bottenberg, Tamò and Singh, 1998; Adati et al., 2008).

Table 5.A1.2. **Parasitoids and entomoviruses attacking the podborer *Maruca vitrata* in West Africa**

Parasitoids	Status	Stage attacked*	Reference
Hymenoptera, Trichogrammatidae			
Trichogrammatoidea eldanae	Indigenous	Egg	Arodokoun et al. (2006)
Hymenoptera, Eulophidae			
Tretrastichus sp.	Indigenous	Pupa	Usua and Singh (1978)
Hymenoptera, Braconidae			
Apanteles taragamae	Introduced	Larva	Srinivasan et al. (2007)
Bassus bruesi	Indigenous	Larva	Arodokoun et al. (2006)
Bracon sp.	Indigenous	Larva	Arodokoun et al. (2006)
Braunsia sp.	Indigenous	Larva	Usua and Singh (1978)
Braunsia kriegeri	Indigenous	Larva	Arodokoun et al. (2006)
Dolichogenidea	Indigenous	Larva	Arodokoun et al. (2006)
Phanerotoma sp.	Indigenous	Egg-larva	Usua and Singh (1978)
Phanerotoma leucobasis	Indigenous	Egg-larva	Arodokoun et al. (2006)
Pristomerus sp.	Indigenous	Larva	Arodokoun et al. (2006)
Testudobracon sp.	Indigenous	Larva	Arodokoun et al. (2006)
Diptera: Tachinidae			
Aplomya metallica	Indigenous	Larva	Agyen-Sampong (1978)
Cadurcia sp.	Indigenous	Larva	Arodokoun et al. (2006)
Nemorilla maculosa	Indigenous	Larva	Srinivasan et al. (2007)
Pseudopetichaeta laevis	Indigenous	Larva	Usua and Singh (1978)
Thecocarcelia incedens	Indigenous	Larva	Agyen-Sampong (1978)
Thelairosoma palposum	Indigenous	Larva	Usua and Singh (1978)
Entomoviruses			
Baculoviridae MaviMNPV	Introduced	Larva	Lee et al. (2007)
Cypoviridae MaviCPV	Indigenous	Larva	Tamò et al. (2003)

Source: Adapted from Tamò et al. (2012).

Table 5.A1.3. **Non-pest arthropods associated with cowpeas**

Families containing natural enemies of cowpea pests	Generalist predators	Bees that forage on cowpea flowers
Braconidae	Phytoseiid mites	Honey bees (*Apis mellifera andonsonii*)
Chalcididae	Coccinellid beetles	Carpenter bees (*Xylocopa* sp)
Encyrtidae	Staphilinid beetles	Digger bees (*Anthophora* sp)
Eulophidae	Mantodea	Bumble bees (*Bombus* ssp)
Ichneumonidae	Formicid ants	Leaf-cutting bees (*Megachile* spp)
Pteromalidae	Anthocoridae bugs	
Scelionidae	Spiders	
Tachinidae		
Trichogrammatidae		

Annex 5.A2. Biotechnological developments

Biotechnological approaches in cowpea improvement

The goal of cowpea breeding programmes is to develop consumer-preferred varieties with high yield and resistance to biotic and abiotic constraints to production. Traditional plant-breeding approaches to cowpea improvement have had many successes over the last 30 years. Recent figures from the Food and Agriculture Organization's statistics (FAOSTAT) show an impressive increase in the productivity of cowpea globally. Three principal methods are used in breeding the self-pollinating cowpea: pedigree, mass selection and single seed descent. The pedigree method, often with slight modifications, is the one most frequently used. Selections are based largely on the main character of interest, for example, resistance to the parasitic weed *Striga*. Detailed data on maturity, time to flower, growth habit, and grain and fodder yields are collected and the most promising single plants selected for advancement. Other traits of interest are selected for as well, including seed colour, seed texture, seed size and leaf yield. The relative importance of these traits varies with the particular breeding programme. For example, leaf yield is more important in eastern and southern Africa while west and central African breeding projects lay more emphasis on grain and fodder yields.

Varieties are available that can yield more than 1 tonne/ha. Over the years, improvements have resulted in more than a doubling of the average yield of the crop, from about 200 kg/ha to about 500 kg/ha. However, even this still-modest level of productivity can only be guaranteed if one or two insecticide sprays are applied.

Unfortunately, there are no utilisable resistance genes for post-flowering insect pests in the cowpea genome. There is little prospect for genetic improvement of cowpea by wide-crossing. Cowpea is extremely well-isolated from other *Vigna* species that might provide sources of resistance genes. Many efforts have sought to create viable wide crosses between cowpea and its nearest relatives, but the gulf has proven too wide. For example, it is known that resistance to some insects such as the legume podborer, *M. vitrata*, exists in a distant relative of cowpea, *V. vexillata*, but interspecific genetic barriers prevent hybridisation. What is true for *M. vitrata* is also true for the cowpea bruchid, and for pod-sucking bugs and thrips. Lack of resistance genes is a major bottleneck that limits the success of conventional cowpea breeding. Biotechnological approaches to finding these genes outside the cowpea genome and transferring them into cowpea may progress cowpea improvement. Given the successes with other crops such as maize, tomatoes, sweet potato and cotton, biotechnological approaches to introduce insect resistance and other traits are being explored for cowpea.

Improved cowpeas developed by using biotechnologies

The first reported use of genetic transformation in cowpeas was conducted by Garcia and colleagues (García, 1986; García et al., 1987) using *Agrobacterium tumefaciens* as the gene vector and although antibiotic-resistant callus was obtained, no whole plants were regenerated. Later, mature de-embryonated cotyledons were used as target tissues for gene transfer (Muthukumar et al., 1996). The authors obtained transgenic plants after selection on the antibiotic, hygromycin. However, transmission of the transgenes to the

next generation could not be demonstrated. When the particle gun was used to deliver genes to cowpea, it was found that they were transmitted to only a small proportion of the progeny and that there was no evidence for stable integration of the transgenes (Ikea et al., 2003). A very promising regeneration and transformation system was described by Kononowicz et al. (1997) and although not pursued at the time, it formed the basis of a system that turned out to be reproducible and that obeys Mendelian rules of inheritance (Popelka et al., 2006). Critical features of this system include suitable explants from cotyledonary nodes or embryonic axes and a tissue culture regime without auxins in the early stages, but which includes a cytokinin at low levels during shoot initiation.

There are now several reports showing experimental evidence for reproducible gene transfer to cowpea, including genes for podborer (Higgins et al., 2012), cowpea weevil (Solleti et al., 2008) and for weed control (Citadin, Cruz and Aragão, 2013) as well as a range of model genes to evaluate the technology (Citadin, Cruz and Aragão, 2013; Behura et al., 2014).

The first insect resistance trait being tested using biotechnology is against the legume podborer, *Maruca vitrata*. The cowpea podborer belongs to the Pyralidae, the family to which the European corn borer (ECB) belongs. ECB, a major pest of maize in the eastern United States, can be controlled by means of maize hybrids genetically engineered to express the *cry1Ab* gene from *Bacillus thuringiensis* (often referred to as *Bt*). In the US Corn Belt, about one-quarter of maize now carries the *cry1Ab* gene. The protein product of this gene has been shown to be toxic to *M. vitrata* when fed in the diet (LC_{50}=0.03 μg/g diet) (Srinivasan, 2008). Accordingly, genetic transformation of cowpea to express the *cry1Ab* protein has the prospect of imparting *M. vitrata* resistance. The African Agricultural Technology Foundation (AATF) based in Kenya is implementing a programme to develop genetically engineered maruca-resistant cowpeas. The bred lines contain the *cry1Ab* gene, with the *nptII* gene used as selectable marker. Being under testing phase, some varieties are expected to reach the African market around 2017 (AATF, n.d.).

Another constraint that cannot be adequately addressed through conventional breeding is resistance to cowpea weevil. While it is true that there are cowpea cultivars derived from the landrace TVu2027 with moderate resistance to cowpea weevil, this resistance has already been incorporated into many cowpea varieties and has been widely disseminated, both in Africa and beyond. It now appears that there are populations of cowpea weevil that can overcome this resistance. Numerous genes have the potential to confer resistance to the cowpea weevil if transferred into cowpea and expressed in the seed. The most advanced of these involves transferring an α-amylase inhibitor (αAI) gene from common bean into cowpea. The αAI protein protects common bean seeds against cowpea weevil and certain other bruchids, though not against the common bean weevil. When αAI was linked to a strong seed-specific promoter and transferred into garden pea using gene technology, the garden pea seeds, which are normally susceptible to cowpea weevil, proved to be highly resistant (Shade et al., 1994). By transferring the common bean αAI gene into cowpea and expressing it in the seeds, it should be possible to introduce a new source of weevil resistance into cowpea. However, some uncertainty hangs over this undertaking as the αAI protein may not be produced in the recipient plant exactly as it is in the donor parent. This has been observed with αAI expressed in garden peas. The αAI protein from garden peas had small mass difference from that of the protein from common bean, a difference probably due to a variation in the degree of post translational modification in the recipient species. The possibility that this variant

protein – which still inhibits insect α-amylase and blocks weevil growth and development – might cause toxicity or allergenicity in consumers of the transformed seed has to be addressed (Prescott et al., 2005), although in a recent comprehensive study this was considered to be unlikely (Lee et al., 2013).

In those cropping areas where cowpea is grown as a sole crop, it could be desirable and feasible to control weeds using a herbicide. It was recently shown that a biotechnological approach could be used to introduce tolerance to a Group B herbicide into cowpea (Citadin, Cruz and Aragão, 2013). This could open the way to a no-tillage farming system for cowpea.

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Part III:

Biology of trees

Chapter 6.

Eucalyptus (*Eucalyptus* spp.)

*This chapter deals with the biology of eucalyptus tree (*Eucalyptus spp.*). It contains information for use during the risk/safety regulatory assessment of genetically engineered varieties intended to be grown in the environment (biosafety). It is focused on those Eucalyptus species and hybrids which are planted commercially and expected to be the subjects of possible genetic modification. The chapter provides elements of taxonomy; centre of origin; domestication and cultivation practices; crop improvement; morphological characters; reproductive biology including sexual/asexual reproduction; pollination and seed dispersal; genetics; abiotic interactions with nutrients, metals, temperature, water, salinity and other stresses; pests and pathogens; weediness; natural and manipulated hybridization; and health considerations.*

This chapter was prepared by the OECD Working Group on the Harmonisation of Regulatory Oversight in Biotechnology, with Australia as the lead country. It was initially issued in August 2014. Information on the main eucalypt species and their hybrids used in plantations in the world (Table 6.1) was updated in January 2016.

Introduction

Eucalyptus is a diverse genus of flowering trees (and a few shrubs) that belongs to the angiosperm family Myrtaceae. The genus includes more than 700 species, most being endemic to Australia. In that continent they are found in a range of different environments, from the dry hot interiors to the cold temperate regions in the south-east, invariably constituting the dominant large plants in forests. Colloquially, many species in Australia are known as "gum trees", a term which refers to the sticky thick sap which exudes from their stems if the bark is broken. A few species are native to Papua New Guinea and Indonesia, and one is native to the Philippines.

In the last 200 years, many of these species have been introduced as exotics in other countries around the world. Most of these are grown in large commercial plantations in the tropics and subtropics, these plantations being particularly prominent in South America, North America, southern Europe, Africa, the Middle East, the People's Republic of China (hereafter "China") and the Indian subcontinent. Wood from *Eucalyptus* is used for the extraction of pulp and timber for building as well as raw material for biofuel production, while some of the organic compounds derived from its leaves have medicinal and insecticidal properties.

The purpose of this chapter is to present information which may be of direct relevance to the assessment of the risks/safety of genetically engineered *Eucalyptus*. Genetically engineered plants are produced by the transformation of one or more genes into their genomes, these genes being selected to confer a desired trait upon the plant. Potentially, the inserted genes and associated traits could affect the health and safety of humans (and animals), as well as the environment. These risks need to be assessed before any such plant is released for cultivation. Information in this chapter includes the reproductive biology, genetics, hybridisation, ecology, allergens and toxins, beneficial chemical products and breeding of *Eucalyptus*.

Those *Eucalyptus* species that are planted commercially are expected to be the subjects of genetic modification. The most important of these species are *Eucalyptus grandis*, *E. urophylla*, *E. pellita*, *E. globulus*, *E. nitens*, *E. dunni*, *E. camaldulensis*, *E. tereticornis* and *E. saligna*, and the hybrids *E. urophylla* x *E. grandis*, *E. camaldulensis* x *E. grandis* and *E. globulus* x *E. nitens*. As plantations of hybrids are becoming increasingly common, it is possible that such plants will form the core of future genetically engineered *Eucalyptus*.

As the centre of *Eucalyptus* diversity, Australia produced much of the early-published research on the biology and ecology of these plants, and a corresponding emphasis on Australian material may be found in parts of this chapter. However, with the increasing commercial importance of *Eucalyptus* worldwide, there has also been considerable output by groups in South America, South Africa and Japan. The present chapter therefore draws extensively on excellent and comprehensive reviews produced by these groups. Information from other world regions has also been included where possible.

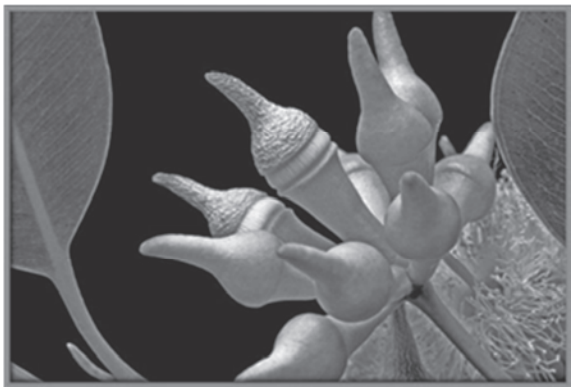
While the volume of research on *Eucalyptus* is large, it has generally focused on a limited number of species. This should be borne in mind when reading general statements relating to *Eucalyptus* biology and ecology in this document.

Taxonomy of species

Classification and nomenclature

The term *Eucalyptus* was coined by the French botanist Charles-Louis L'Héritier de Brutelle in the late 18th century while characterising a specimen brought back from Adventure Bay, Tasmania, on the third expedition of Captain Cook to Australia and the Pacific (Kantvilas, 1996). He made the word from two Greek roots, *eu* and *kalyptos*, meaning "well" and "covered" respectively, the reference being to the operculum, the cap on the flower bud which protects the plant reproductive structures prior to its displacement by the growing stamens (Figure 6.1).

Figure 6.1. **Displacement of *Eucalyptus robusta* operculum by growing stamens**



Source: Courtesy Brian Johnston, 2007.

Eucalyptus is a genus of the Myrtaceae family, a family which is mainly found in countries of the southern hemisphere (Rozefelds, 1996). The Myrtaceae also includes genera such as *Melaleuca*, *Callistemon*, *Psidium* (guava) and *Syzygium* (cloves).

The term "eucalypt" is sometimes used as the common name of the *Eucalyptus* genus. However, it is more accurately used as a term referring to species from a monophyletic group, broadly referred to as the "eucalypt group", which encompasses seven genera: *Eucalyptus*, *Angophora*, *Corymbia*, *Eucalyptopsis*, *Allosyncarpia*, *Stockwellia* and *Arillastrum* (Ladiges, Udovicic and Nelson, 2003). *Eucalyptus* L'Hér. *sensu stricto* is the largest genus of the group.

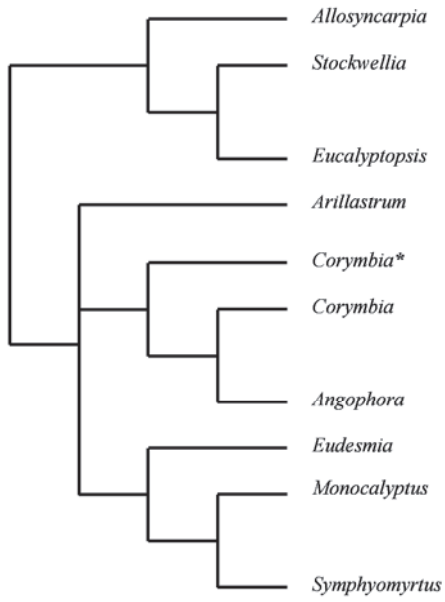
Most *Eucalyptus* species are found in Australia, where it is the dominant biota in mature forests, but species also naturally occur in Papua New Guinea and adjacent islands, Indonesia and the Philippines (one species). Members of this genus are usually long-lived evergreen hardwood plants, varying from shrubs to tall forest trees, a common distinctive feature being the aroma of their oils.

Morphological examination of *Eucalyptus* has given rise to a number of different taxonomic classifications of species. One influential study defined two genera, *Angophora* and *Eucalyptus*, the latter consisting of 7 subgenera (Pryor and Johnson, 1971), while more recently another important classification suggested a single genus (*Eucalyptus*) consisting of 13 subgenera (Brooker, 2000). Important physical characters that have been used in these classifications include the structure of the flower, the shapes of the leaves, and the shapes and sizes of the seeds. Although disagreement concerning

aspects of the taxonomic rank and number of groupings will remain, the three major lineages of *Eucalyptu*s are *Symphyomyrtus*, *Monocalyptus* and *Eudesmia* (Figure 6.2). These three lineages, referred to here as subgenera, contain approximately 450, 110 and 20 species, respectively. *Symphyomyrtus* includes gums, ironbarks and mallees, *Monocalyptus* trees such as jarrah, most of the stringybarks, and the mountain ash, while Bailey's stringybark and Darwin stringybark are members of the *Eudesmia* (Boland et al., 2006). In undisturbed Australian forests it is common to find mixed stands, consisting of one species each of the *Symphyomyrtus* and *Monocalyptus* subgenera (Davidson and Reid, 1980).

Molecular techniques, involving the analysis of specified DNA sequences, have been used to establish phylogenetic relationships within *Eucalyptus*. Sequences which have been examined include the internal transcribed spacer (ITS) and external transcribed spacer (ETS) regions of the nuclear ribosomal DNA, various chloroplast sequences, and the nuclear gene cinnamoyl CoA reductase. These studies have generally confirmed the definition of *Eucalyptus* and the other "eucalypt" genera (*Angophora*, *Corymbia*, etc.) as distinct but closely related entities, and within *Eucalyptus* the division into the subgenera *Symphyomyrtus*, *Monocalyptus* and *Eudesmia* (Ladiges, Udovicic and Drinnan, 1995; McKinnon et al., 2008; Parra-O et al., 2006; Steane et al., 2002, 1999) (Figure 6.2). These basic taxonomic classifications have also been supported by an analysis of the concentration of the metabolite quercitol amongst the eucalypts (Merchant, Ladiges and Adams, 2007).

Figure 6.2. **Simplified phylogeny of the major groups of eucalypts based on both nuclear and chloroplast DNA sequence data**



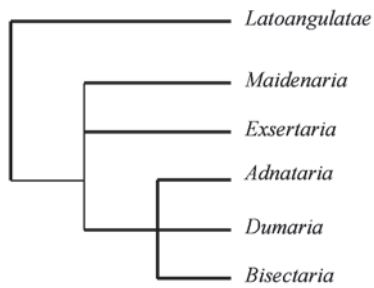
Note: *Alternative grouping for *Corymbia* based on different data sets.

Source: Adapted from Ladiges, Udovicic and Drinnan (1995) and Ladiges, Udovicic and Nelson (2003).

The resolution of phylogenetic relationships within each of the subgenera by the use of molecular techniques has proven more difficult. Examination of variations in chloroplast DNA sequences have shown that they confirm the subgenera with *Eucalyptus*,

but within subgenera such approaches have produced data which do not always correlate with that from morphological studies (McKinnon et al., 2004; 1999). The difficulty in resolving taxonomic relationships within these subgenera is likely due to convergent evolution and hybridisation/introgression between species. At least in the *Eucalyptus* subgenus *Symphyomyrtus*, data from ITS sequences have revealed relationships between the sections of this subgenus which correlate with records of naturally occurring inter-sectional hybrids (Steane et al., 2002). Recently, amplified fragment length polymorphism (AFLP) markers have been used to resolve some of the relationships in the *Maidenaria* of the subgenus *Symphyomyrtus* (McKinnon et al., 2008). Within this section, the AFLP analysis provided both a resolution of the relationships between species for which previous molecular studies had been equivocal, as well as a set of phylogenetic relationships which strongly correlated with those based upon morphology (Figure 6.3).

Figure 6.3. **Phylogeny of the sections within the *Eucalyptus* subgenus *Symphyomyrtus***



Source: Adapted from Ladiges (1997).

The *Eucalyptus* species that have become the focus of major commercial enterprises and biotechnology belong mainly to the subgenus *Symphyomyrtus*. The major morphological identifying features of this lineage are seeds possessing coats consisting of one integument, and flowers with two opercula, the latter being sometimes fused. More specifically, these commercially important species belong to 3 of the 15 taxonomic sections of *Symphyomyrtus*. These sections, and their principle species, are:

- Section Latoangulatae: *E. grandis*, *E. urophylla*, *E. pellita* and *E. saligna*
- Section Maidenaria: *E. globulus*, *E. nitens* and *E. dunni*
- Section Exsertaria: *E. camaldulensis* and *E. tereticornis*

Latoangulatae (or *Transversaria* under the informal classification of Pryor and Johnson, 1971) are characterised by discolorous dorsiventral adult leaves (Boland et al., 2006; Brooker, 2000). Most of the species in this section are native to the mountain ranges of eastern Australia and the adjacent coasts, although a small number are found in the islands to the north of Australia. Plants in the section *Maidenaria* usually have sessile juvenile leaves and oil glands in their bark. They are mainly native to south-eastern Australia. The red gums, native to south-eastern and north-eastern Australia, constitute the section *Exsertaria*, one of their defining features being the petiolate nature of their juvenile leaves.

Origin and cultivation

Centre of diversity and domestication

The earliest fossils of Myrtaceae pollen, dating to the late Cretaceous period (85 million years ago), have been identified in deposits from Colombia, Gabon and Borneo (Muller, 1981). In Australia, the earliest occurrences of pollen fossils from this family date to the Palaeocene (65-55 million years ago), but identifying specimens as belonging to *Eucalyptus* (as opposed to other eucalypt genera such as *Angophora*) has proven more difficult (Martin, 1994). However, pollen from *E. spathulata* has been described from three sites dating to the end of the Tertiary period (Martin, 1988). Fossils of leaves and fruit from plants identified as belonging to the Myrtaceae family have been unearthed from a number of sites in Australia, and dated to various epochs within the Tertiary (Christophel and Lys, 1986; Lange, 1978). Other fossils have been more specifically identified as originating from *Eucalyptus* species. These include the Miocene fossils of leaves and fruit from species which have features similar to extant members of the subgenus *Symphyomyrtus* (Holmes, Holmes and Martin, 1982; Pole et al., 1993).

Fossils identified as coming from plants that may be members of *Eucalyptus* have been described from both Argentina and New Zealand (Frenguelli, 1953; Pole, 1993). At least in the case of the fossils from New Zealand, it has been suggested that they reflect a trans-Tasman migration of species. Although it may not be possible to determine with certainty the place of origin of the first plant that can be defined as *Eucalyptus*, the predominant native distribution of this genus in Australia (together with its modern absence from regions such as New Zealand and South America) has led to Australia being accepted as the likely region of its evolution.

As noted above, *Eucalyptus* is native not only to Australia, but some species are also endemic to the neighbouring islands to the north. These include *E. urophylla* and *E. deglupta* and a small number which occur in both Australia and Papua New Guinea (e.g. *E. alba* and *E. tereticornis*). Within Australia, *Eucalyptus* species are found in nearly all vegetation zones, the only exceptions being the rainforests in the north-east of the continent, the arid interior and the high alpine areas of the south-east. The subgenus *Symphyomyrtus*, which contains the most species, is also the most widespread. As large areas of Australia are prone to drought with infrequent floods, *Eucalyptus* species that come from these areas are adapted to surviving in soils with little available moisture (Morton et al., 2011).

The history of eucalypt introductions and subsequent domestication in exotic environments has been reviewed by Eldridge et al. (1994). Following the first record in Australia in the late 18th century, eucalypts were spread rapidly around the world into countries such as India (*c.* 1790), France (*c.* 1804), Chile (1823), Brazil (1825), South Africa (1828) and Portugal (1829) (Iglesias-Trabado and Wisterman, 2008; Potts, 2004) (Figure 6.4). They were initially introduced as botanical curiosities, but the potential for some species to grow fast was quickly recognised and they became widely planted for fuel wood and timber production. Eucalypts are now found in more than 90 countries (Iglesias-Trabado and Wisterman, 2008), having expanded rapidly in recent decades to total over 20 millions hectares in 2013 (Harwood, 2014)[1].

The majority of plantations consist of only a few eucalypt species and hybrids. Based on visits to the major grower countries and discussions with grower agencies, Harwood (2011) estimated that nine eucalypt species in the subgenus *Symphyomyrtus* (Brooker,

2000), and clonal plantations of various interspecific hybrids among these species, account for 90-95% of the world's planted eucalypts (Table 6.1).

Figure 6.4. ***Eucalyptus globulus* in Hawaii**

Source: Forest and Kim Starr, licensed under CC BY-SA 3.0.

Cultivation and commercial uses

Eucalyptus, together with *Pinus*, are the most important commercially grown tree taxa (Richardson, 1998). Brazil and India have the largest areas of *Eucalyptus* plantations, but significant areas are found in Angola, China, Portugal, South Africa, Spain and Viet Nam.

The wood from *Eucalyptus* can be used for the production of poles and timber boards and beams for building, pallets, crates and furniture. Wood chips and bark particles from trees can be used for mulch, as well as serving as a fuel. More processed products include plywood, chipboards and fibreboards. Australian aboriginals have traditionally used *Eucalyptus* as a source of wood for making didgeridoos and many other artefacts.

Especially in Australia, Brazil, Chile, Portugal and South Africa, the wood from plantation-grown *Eucalyptus* species is used for the production of paper pulp, especially bright photocopy paper (Turnbull, 1999). *Eucalyptus* is the largest single global source of market pulp; it has been estimated that, by the end of 2011, global market pulp production would reach about 65 million tonnes, with about 33 million coming from hardwoods, and 55% of this coming from eucalypts (ICEP, 2011). Brazil also uses significant amounts of *Eucalyptus* wood to produce charcoal for its iron and steel industries (Figure 6.5).

Eucalyptus is renowned for the wide range of organic compounds that it produces. Climatic factors in Australia have likely played a significant role in the evolution of this feature. Originating in environments that are usually rich in sunshine and exposed to periodic rainfall, *Eucalyptus* species frequently conduct photosynthesis year-round, enabling the production of an abundant quantity and variety of carbohydrates and other carbon-based compounds (Orians and Milewski, 2007). From a chemical perspective, these compounds are often low in their nitrogen content, almost certainly a reflection of the poor available nitrogen content of many Australian soils. *Eucalyptus* oils are used in flavourings, fragrances, cosmetics and mouthwash.

Table 6.1. **Nine eucalypt species and their hybrid combinations dominate the world's eucalypt plantations**

Country	Most important species	Approximate range of mean annual temperature (°C) for good growth in plantations	Area in plantations 2012 (M ha)	% of clonal plantations	Rotation length (years)	Coppicing
Argentina, Australia, Chile, China (People's Republic of), Ethiopia, Portugal, Spain	*E. globulus*	9-18	3.0	10	12	Yes
Australia, Chile	*E. nitens*	9-18	0.4	0	12	No
Brazil	*E. urophylla* x *grandis*		4.0		6-7	Yes
China (People's Republic of)	*E. dunnii*	14-22	0.1	0	7	No
	E. grandis	14-25	0.1	80	7	Yes
	E. urophylla x *grandis*		2.0	100	5	Yes
India	*E. camaldulensis* and *E. tereticornis*	18-28 (northern provenances)	2.0	30	4-7	Yes
		13-22 (southern provenances)				
		17-27 (northern provenances)			4-7	Yes
Indonesia	*E. pellita*	20-27	0.3	90	5	Yes
South Africa	*E. grandis*	14-25	0.6	60	7	Yes
	E. dunnii	14-22				
	E. urophylla x *grandis*					
Thailand	*E. camaldulensis and hybrids*	18-28 (northern provenances)	0.3	90	4	Yes
		13-22 (southern provenances)				
Viet Nam	*E. urophylla*	18-28	0.4	10	6-8	Yes
	E. camaldulensis	18-28 (northern provenances)				
		13-22 (southern provenances)				
Uruguay	*E. grandis*	14-25	0.7	20	8	Yes
	E. dunnii	14-22				No
Other countries	Various, including	Various	7	Various	Various	Various
	E. saligna	14-23				
TOTAL			**20**			

Source: Adapted from Harwood (2014; 2011).

Figure 6.5. ***Eucalyptus* plantation in Brazil**

Source: Cássio Abreu, licenced under CC-BY-2.0.

Worldwide, the most important plantation species are the pure species *E. camaldulensis*, *E. grandis*, *E. saligna*, *E. nitens* and *E. globulus*, and the hybrids *E. urophylla* x *E. grandis*, *E. camaldulensis* x *E. grandis* and *E. globulus* x *E. nitens* (Martin, 2003). In Australia, *Eucalyptus* species make up approximately 95% of broadleaf plantation species, over half of the *Eucalyptus* estate being composed of *E. globulus* (540 000 ha) and one-quarter by *E. nitens* (236 000 ha) (Gavran, 2012). Due to its adaptations to soils with low fertility, the hybrid *E. urophylla* x *E. grandis* has become the most common tree in Brazilian plantations, whereas in drier regions, hybrids of *E. camaldulensis* are favoured (Goncalves et al., 2008). In Brazil, the yield of wood products, mainly pulp, from *Eucalyptus* plantations has increased from 12 m^3/ha/year to 40 m^3/ha/year in 30 years (Campinhos, 1999), while in India, eucalypt plantations may be able to meet that country's demand for paper and pulp (Lal, 2010). Probably over 25% of the world's eucalypt plantation area involves interspecific hybrids, because these dominate the plantations of two of the biggest growing nations, Brazil and China, and are important in several other countries such as South Africa (Harwood, 2014)[2].

Generally, the rotation period in *Eucalyptus* plantations is 6-14 years, with the density ranging from 400 trees to 1 100 trees per hectare (Martin, 2003). Rotation periods are even lower for some hybrids: from four to seven years (Harwood, 2014)[3]. These rotation times are significantly less than for other plantation species, especially species of *Pinus*, *Betula* and *Picea* (Campinhos, 1999). The ability to coppice plants (cut back to their stumps to allow fresh regeneration) in a plantation is advantageous because the costs of re-establishment are substantially reduced. Moreover, at least the first stages of regrowth are usually faster than growth from seed, and the resulting trunks straighter. *Eucalyptus* is readily amenable to coppicing, and most *Eucalyptus* plantations around the world are managed by this method (Matthews, 1991). In Brazil, *E. grandis* plantations are usually grown on coppice rotations of between five and ten years (Turnbull, 1999), and six to seven years for *E. urophylla* x *E. grandis* plantations (Table 6.1). It is normally possible to coppice *Eucalyptus* many times, but, while the yield from the first regrowth is occasionally greater than that of the original plant, the yields from subsequent such treatments are lower. Typically, the forest will be replanted with new seedlings of a genetically improved clone or seed crop newly derived from the breeding programme.

Eucalyptus plantation management has benefited from the application of the 3-PG model, a generalised forest carbon allocation model. Studies in Brazil and Portugal have demonstrated the usefulness of the model in predicting the growth patterns of stands, including characters such as height (Almeida, Landsberg and Sands, 2004; Rodriguez-Suarez et al., 2010).

The narrow genetic base of *Eucalyptus* plantations with clonally propagated elite trees (see below) has raised concerns about increasing vulnerability to insect or pathogen attack. After more than 30 years of clonal plantations in Brazil, however, this concern has proven largely overestimated and no documented cases exist of increased pest or pathogen attack to eucalypt clonal forests. Reasons that in practice mitigate this risk include: 1) every company recommends 2-5 new clones every 3-4 years so that a complete replacement of the clone portfolio will take place every 10-15 years; 2) each company plants 8-15 clones at any time so that the contribution of a single clone to the total planted area will be relatively small, and problematic clones, if they occur, can be rapidly removed with small relative damage; 3) clonal plantations are established in clonal blocks of 5-50 hectares with a single clone per block in a mosaic format so as to avoid a neighbourhood of blocks with the same clone; 4) breeding programmes exploit large amounts of genetic diversity so that output clones have very diverse genetic backgrounds; 5) clonal trials prior to final recommendation for commercial use adopt rigorous screening procedures for the common pathogens, since one of the major advantages of clonal deployment is the large-scale plantation of highly resistant trees (Grattapaglia et al., 2012). Additionally, in most plantations in Brazil, the maintenance of extensive areas of native vegetation contiguous to the *Eucalyptus* forests has proven an effective measure for the control of insects, as this vegetation is preferred by native birds which feed on insects. It has been recommended that plantations have a range of clones to both diminish the risk of low genetic variability and enable adaptation to changed environmental conditions (Campinhos, 1999). Percentages of clonal plantations estimated for some countries in 2012 are reported in Table 6.1.[4]

Crop improvement

Breeding

Breeding programmes in *Eucalyptus*, as with most common crop plants, have focused on the crossing of relevant elite lines containing desirable traits. The open pollination of flowers (i.e. pollination through natural mechanisms) is often used to produce hybrid seed, but controlled (hand) pollination is also practised (Horsley, Johnson and Myburg, 2010; Suitor et al., 2008). Open pollination is obviously easy, but suffers from the disadvantage that it will usually result in the presence of undesired self-pollinated individuals. Controlled pollinations have traditionally involved multiple visits to flowers, emasculation, and wounding or cutting of the stigma or style to enhance its receptivity to germination of pollen and the formation of pollen tubes. Such techniques are both time-consuming and costly, but so-called one-stop pollination, involving a single visit to a flower, has been developed for some species (Harbard, Griffin and Espejo, 1999). At least for *E. globulus*, it is possible to obtain successful fertilisation by the pollination of immature styles prior to flower dehiscence (Trindale et al., 2001), and also by cutting the style but not concurrently utilising emasculation (Patterson et al., 2004). This technique has now been optimised for several eucalypt species and given the term artificially induced protogyny; it has led to a significant advance in the ability to generate large quantities of seed from controlled crosses (de Assis, Warburton and Harwood, 2005).

The development of techniques for the clonal (asexual) propagation of plants has meant that plantations in countries such as Brazil and India now consist largely of clonal plantations, the sowing of seeds having been largely abandoned (Eldridge et al., 1994; Lal, 2010). Clonal propagation can be conducted by the use of rooted stem cuttings, but this technique has proven unsuitable for a number of commercially important species, either because of the difficulty in obtaining roots or of the large number of rooted plants which have developmental problems. However, cuttings taken from cotyledons, shoot apices and axillary sprouts are much easier to manage (de Assis, Fett-Neto and Alfenas, 2004; Le Roux and Van Staden, 1991). The mini-cutting technique, now widely adopted in Brazil and some other countries, currently represents the most efficient way to clonally propagate eucalypts (de Assis, 2011) (Figure 6.6). Experiments in Africa with terminal shoots of *E. urophylla* x *E. grandis* have demonstrated that root development is superior if they come from juvenile trees (at least during the dry season), but shoots derived from the regrowth of coppiced trees root equally well regardless of the age of the felled tree (Mankessi et al., 2011).

Figure 6.6. **Mass propagation of *Eucalyptus* seedlings**

Source: Balaji Kasirajan, licenced under CC BY-SA 3.0.

Clones of *Eucalyptus* can also be generated by somatic embryogenesis, a technique which allows the formation of embryos from somatic or haploid cells, avoiding the need for gamete fusion. Somatic embryogenesis has been used to propagate *E. citriodora*, *E. dunnii*, *E. grandis*, *E. nitens* and *E. globulus* (Pinto et al., 2002) at the experimental level. For *E. globulus*, a protocol for somatic embryogenesis has been developed that may enable the industrial production of such tissue (Pinto et al., 2010), although no operationally viable protocol exists. In that plant, it is apparent that induction of somatic embryogenesis is under additive genetic effects, in particular the so-called general combining ability effect (Pinto et al., 2008). Importantly, micro-propagated tissues and embryos are amenable to protocols for the transformation of genes, such as those using *Agrobacterium tumefaciens*.

In Brazil, trees with desirable traits, such as above-average rates of growth, are selected in screenings of individuals and cloned by using one of these techniques. However, at least in some cases, further enhancement of populations through the selection of individuals from already improved trees is proving difficult, and techniques such as artificial hybridisation may be useful (Fonseca et al., 2010). This latter technique involves the controlled crossing of individuals and the field evaluation of the progeny.

Modern molecular techniques, such as measuring the expression levels of genes known to influence a trait, may prove useful in selecting the individual trees for clonal propagation. Even for trees which are the product of natural or traditional breeding, such techniques can be employed to measure the success and/or levels of outcrossing (Gaiotto, Bramucci and Grattapaglia, 1997). In order to compare the expression of genes between species, especially when employing the polymerase chain reaction (PCR), a reference gene is needed for normalisation. Genes suitable for this role have been identified for *E. globulus*, and should prove useful in other species (Almeida et al., 2010b).

Genetic linkage maps, based on RFLP, RAPD and AFLP molecular markers, have been constructed for species such as *E. grandis*, *E. urophylla* and *E. globulus* (Gan et al., 2003; Grattapaglia and Kirst, 2008; Grattapaglia and Sederoff, 1994; Myburg et al., 2003). A microsatellite map, covering at least 90% of the genome of *Eucalyptus* and containing over 230 mapped loci, has also been published (Brondani et al., 2006), which was recently significantly expanded by using much higher throughput marker technologies (Hudson et al., 2012b). Microsatellite markers have been widely used for genotyping species of *Eucalyptus*, especially those in the subgenus *Symphyomyrtus* (Faria et al., 2011; Kirst et al., 2005). These latter markers were based on the sequences of existing expressed sequence tags (ESTs), and it is expected that they will be useful in differentiating individuals and become part of work which necessitates clone fingerprinting and the testing of parentage. Future marker-assisted breeding programmes will likely use a range of different molecular markers, including those arising from high throughput techniques such as diversity arrays technology (Sansaloni et al., 2010) or genotyping by sequencing (Faria et al., 2012).

Genetic modification

The success of *Eucalyptus* as a plantation crop has meant that it has been the subject of research aimed at improving some of its associated traits by the use of genetic engineering. In particular, the role of *Eucalyptus* in the paper industry has focused attention on improving traits of productivity and wood quality, for which the sensitivity of many commercial species and hybrids to cold temperatures has been a major target. Constitutive overexpression and controlled stress induction of C-repeat binding factor (*CBF*) genes in *E. grandis* x *E. urophylla* has resulted in the isolation of "freeze-tolerant" plants (Hinchee et al., 2009; Navarro et al., 2011). The US Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS) has issued a number of permits for the field testing of *Eucalyptus* trees engineered with the *CBF* gene, this process involving the preparation of environmental assessments (APHIS, 2012a; 2012b).

These plants have demonstrated tolerance to temperatures of -9ºC. The products of the *CBF* genes are transcription factors which activate a stress responsive pathway by binding to specific *cis*-acting regulatory sequences. In a different approach, cold stress tolerance in *E. saligna* has been addressed by the transformation into plants of the Δ^1-pyrroline-5-carboxylate synthetase gene (*P5CS*, coding for a key enzyme in proline biosynthesis) from *Vigna aconitifolia* (Dibax et al., 2010).

Other traits of *Eucalyptus* that have been subject to modification by genetic engineering include responses to a range of biotic and abiotic stresses, constitution of the endogenous essential oils and the biosynthesis of lignin. Table 6.2 summarises the major genetic modifications of *Eucalyptus* in published literature.

Table 6.2. **Genetic modifications of *Eucalyptus***

Plant	Gene inserted	Trait	Reference
E. urophylla	*RS-AFP2* from radish	Disease resistance	Ouyang et al. (2012)
E. globulus	*Choline oxidase (codA)* from *Arthrobactor globiformis*	Salt stress and/or drought tolerance	Matsunaga et al. (2012); Yu et al. (2009; 2012a)
E. globulus	*des9* from cyanobacteria	Low temperature	Japan Biosafety Clearing-House (2011)
E. globulus	Antisense *LIM domain transcription factor*	Decrease in lignin content	Shimazaki et al. (2009)
E. grandis x *E. urophylla*	*CBF* from *Arabidopsis* and *E. gunnii*	Cold tolerance	Hinchee et al. (2009); Navarro et al. (2011)
E. saligna	*P5CS* from *Vigna aconitifolia*	Cold tolerance	Dibax et al. (2010)
E. camaldulensis	*Mangrin* from the mangrove plant *Bruguiera sexangula*	Salt tolerance	Lelmen et al. (2010)
E. camaldulensis	*Limonene synthase* from *Perilla frutescens*	Monoterpene composition	Ohara et al. (2010)
E. camaldulensis	Antisense *LIM domain transcription factor*	Decrease in lignin content	Kawaoka et al. (2006)
E. camaldulensis	*choline oxidase (codA)* from *Arthrobactor globiformis*	Salt stress and drought tolerances	Kikuchi et al. (2009)
E. camaldulensis	*DREB1A* from *Arabidopsis*	Salt stress and drought tolerances	Hibino (2009)
E. camaldulensis	*Mangrin* from the mangrove plant *Bruguiera sexangula*	Salt stress and drought tolerances	Lelmen et al. (2010); Yu et al. (2012b)
E. grandis x *E. urophylla*	Radish plasma membrane aquaporin gene	Drought tolerance and water use efficiency	Tsuchihira et al. (2010)
E. grandis x *E. urophylla*	Antisense *cinnamyl alcohol dehydrogenase (CAD)*	Decrease in lignin content	Tournier et al. (2003); Valerio et al. (2003)

Transformation of *Eucalyptus* has been achieved both through biolistics and the use of *Agrobacterium tumefaciens*. In regard to biolistics, for example, zygotic embryos of *E. globulus* have been stably transformed after biolistic delivery with linear DNA constructs (Serrano et al., 1996). However, *Agrobacterium* mediated transformation was used to generate all of the genetically modified plants in Table 6.2. Other reports pertaining more generally to *Agrobacterium* transformation relate to *E. camaldulensis* (Mullins et al., 1997), *E. globulus* (Moralejo et al., 1998), *E. grandis* x *E. urophylla* (Gonzalez et al., 2002; Machado et al., 1997), *E. occidentalis* (Southerton, 2007) and *E. tereticornis* (Prakash and Gurumurthi, 2009). Usually explants of shoots, leaves and cotyledons are used for *Agrobacterium* transformation.

The isolation and characterisation of genes associated with a specific trait is instrumental in the understanding of the molecular basis of that trait, as well as providing a pool of clones from which promising members can be selected for transformation (Harakava, 2005). For *Eucalyptus*, many endogenous genes for transformation are likely to come from the screening of EST and cDNA libraries for genes involved in fundamental (and commercially important) developmental processes, such as the biosynthesis of lignin. Genes can be expressed with their endogenous promoters, engineered to be expressed with tissue-specific, temporal-specific or constitutive promoters, or appropriately manipulated and inserted in transformation vectors for the silencing of their expression.

Morphology

Plant morphology

Eucalyptus species are almost all broad-leaved evergreens, but in northern Australia there are a small number of deciduous or semi-deciduous species which will lose their leaves if severely water stressed by the end of the dry season. Most prominent among these latter plants is *E. platyphylla*, commonly known as the poplar gum or cabbage gum.

Species of *Eucalyptus* vary greatly in height, from less than 1 m to over 90 m. In Australia, the larger species constitute the dominant visual flora of most landscapes (Williams and Brooker, 1997). *E. diversifolia*, one of the smaller species, may grow to only 40 cm in the windy environments along the southern coast of Australia, while *E. regnans* (mountain ash), which is native to the south-eastern Australian mainland and Tasmania, reaches at least 90 m, with reports of individuals of over 100 m (*http://gianttrees.com.au*; Hickey, Kostoglou and Sargison, 2000). The diameters of trees of this latter species can reach well over 5 m (Figure 6.7).

Figure 6.7. **Diversity of form amongst eucalypt species**

A. A tall forest tree, *E. grandis* can grow to 45-50 m in height

B. *E. macrocarpa*, a small mallee grows up to 4 m in height

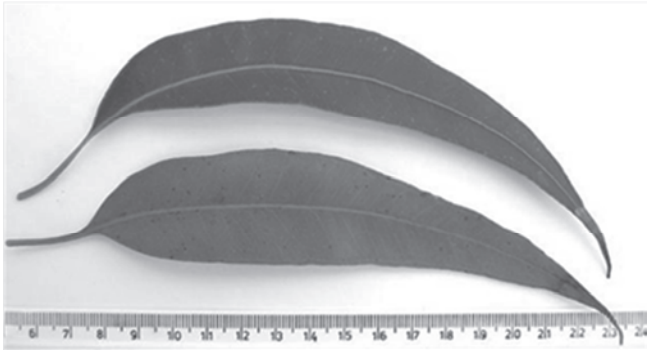
Source: Courtesy Alison Wardrop, OGTR.

The leaves of *Eucalyptus* are usually asymmetrical about the central midrib, a feature common in many tree genera (Figure 6.8). In most species, the leaves of adult trees hang vertically, while those of juvenile trees are near horizontal. This feature of adult trees is responsible for the large amount of light which reaches the floor of *Eucalyptus* forests, especially in comparison to broad-leaf and conifer forests.

The developmental stage at which the shift in leaf angle from horizontal to vertical occurs varies between species. Generally, leaves that are vertical have almost the same colour and morphology on both sides; conversely, these traits are much less common in species with horizontal leaves (King, 1997). Vertical leaves have the advantage of decreasing the interception of light in the middle of the day, thus decreasing the loss of water by transpiration when the day is hottest. In the case of the vertical hanging leaves of *E. globulus*, there is no preference for either side of the leaves receiving most of the incident radiation. On the other hand, the horizontal leaves of young plants intercept

greater amounts of radiation than vertical leaves, and as such their leaves are almost certainly exposed to greater transpirational water loss (James and Bell, 2000). Nevertheless, the horizontal nature of these leaves may be beneficial in increasing photosynthesis and promoting the growth of young plants and the formation of a mature canopy.

Figure 6.8. **Leaves of *E. grandis***



Source: SelecTree (2016). This citation has been added for update in January 2016.

In Australia, as might be expected, species with adult vertical leaves predominate in the dry interior, but surprisingly they are also very common in the wetter forests of the south-eastern part of the mainland and in Tasmania. The explanation for this latter phenomenon may be related to advantages in accessing radiation in winter when the sun is lower in the skies (especially at higher latitudes), and in reducing the effects of cold night temperature induced photoinhibition (Ball, Hodges and Laughlin, 1991; King, 1997).

The barks of *Eucalyptus* species are usually classified as either "rough" or "smooth". Rough-barked species (invariably large trees) have thick barks which break and are lost all year round, the surface beneath being essentially bark identical to that which is lost. The bark of smooth-barked species seasonally sheds, partially detaching and then finally falling off trunks in long strips. The exposed surface of the trunk is often characterised by the scribbles left by insects, leading to the term "scribbly gums" being loosely applied to such species. *E. deglupta*, a native of Papua New Guinea, the adjacent islands, and Mindanao in the Philippines, has brightly coloured smooth bark of shades of yellow, orange, red, green and brown; it is commonly known as the Rainbow *Eucalyptus* (Figure 6.9).

Some species of *Eucalyptus* produce a single stem from the time of germination, while others are characterised in their juvenile stage by several horizontal and/or oblique shoots, one of which will later become the major vertical stem. In the growing crown, the major branches seemingly compete with each other for prominence, eventually establishing the structure of the mature crown. A tree can remain in this mature phase for decades, and even more than a century (Florence, 1996).

Morphological characters that are used to differentiate *Eucalyptus* species, and the subspecies within a species, include the structure of the flower (number of capsules per umbel, size of the capsule, number of ribs per capsule), and the size and shapes of leaves (both in seedlings and adult plants). Leaf characteristics have proven particularly useful in differentiating two or more species that are nearly identical in other visible traits. Not

only do leaves show variation between species, but often show both intraspecific and interspecific clinal variation[5] (Phillips and Reid, 1980; Potts and Reid, 1985). At least in some cases, if not most, this clinal variation is under genetic control.

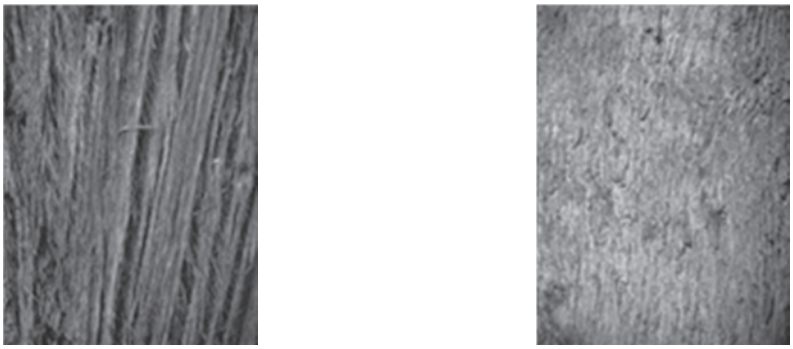
Figure 6.9. **Barks of *Eucalyptus* species**

A. Smooth-barked species

E. camaldulensis | *E. rossii* (scribbly gum) | *E. deglupta* (Rainbow eucalypt)

B. Rough-barked species

E. macrorhyncha (red stringybark) | *E. angophoroides*



Sources: (A): left: courtesy Paul Venter, July 2006, http://en.wikipedia.org/wiki/Image:Euc_cam03.jpg; centre: courtesy Alison Wardrop, OGTR; right: Mann Jess, licenced under CC BY-SA 3.0. (B): left: courtesy Alison Wardrop, OGTR; right: benjamint444, licenced under CC BY-SA 3.0.

Traditionally, observation of the existence of hybrid plants, and the frequency of hybridisation, has depended on the examination of morphological characters, the expectation being that hybrid individuals will possess a mixture of characters and/or characters intermediate between those of the pure bred progenitors. Usually hybrid plants are found where two closely related *Eucalyptus* species overlap in their respective habitats, the presence of these plants indicating the plasticity of the reproductive barriers between the species. However, it is now generally accepted that relying on morphological characters alone will invariably underestimate the number of hybrid plants, and molecular markers give a more accurate estimate of the levels of hybridisation (Field et al., 2009). As with other plants, the ability of *Eucalyptus* species to hybridise depends upon the flowering times of the potential parents (Barbour et al., 2002). Generalised flowering times in Australia of the major plantation species have been compiled and summarised by Potts, Barbour and Hingston (2001; also see Eldridge et al., 1994).

Some species show distinct intraspecific variation. For instance, there are four subspecies of *E. globulus*, each defined not only by their distinct morphology but, in their native Australia, by separate yet overlapping geographical locations (Jones et al., 2002). Likewise, *E. diversifolia* can be divided into three morphologically separate subspecies (Wright and Ladiges, 1997). In many cases, intraspecific variation is at least partly due to the definite isolation of populations, these often being separated by large distances (Shaw, Potts and Reid, 1984). Such variation is also reflected in traits such as pest and pathogen resistance (Guimaraes et al., 2010; O'Reilly-Wapstra, McArthur and Potts, 2002).

Reproductive morphology

The inflorescences of *Eucalyptus* species are formed in the axils of leaves. Most species have a simple inflorescence, but some possess a compound inflorescence which may be either lateral or terminal. Initially, the inflorescence is surrounded by bracts, which are shed to reveal the juvenile bud or buds. Each bud, the progenitor of a flower, develops into a cup-shaped structure, with this process often occurring over at least a two-year period prior to the actual commencement of flowering. Although some species, such as *E. globulus*, have a single bud per inflorescence, most species have higher odd numbers of buds arranged in a cyme, the most common numbers being three and seven. In cymes with only a small number of buds, the pattern of branching is dichasial, but with increasing numbers of buds, an initial dichasial system is usually replaced by a monochasial system (Carr and Carr, 1959). Some species, such as *E. pauciflora*, can have inflorescences with over 50 buds. For many species, a single tree branch will concurrently contain inflorescences still surrounded by bracts, immature buds, flowers and seed pods, thus representing the entire range of developmental stages in flowering (Florence, 1996) (Figure 6.10).

Figure 6.10. ***Eucalyptus tereticornis* buds, capsules, flowers and foliage**

Source: Ethel Aardvark, licenced under CC BY-SA 3.0.

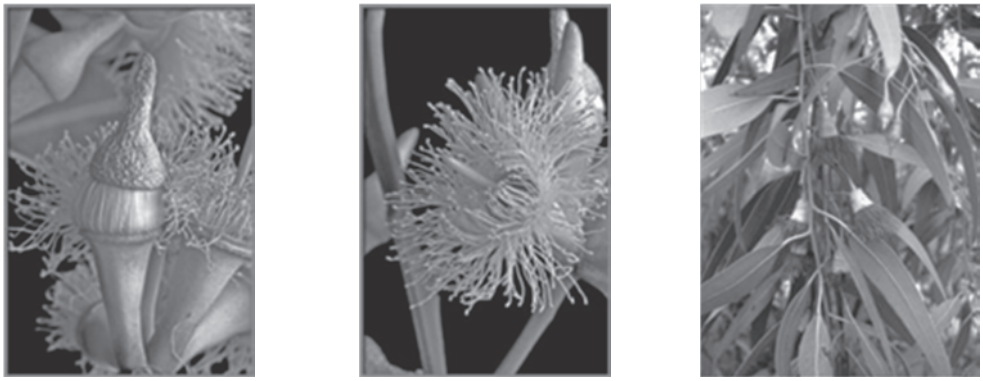
The flowers of most *Eucalyptus* species have functional male and female structures. The stamens and style are covered by a cap termed the operculum, which is forced off the cup-shaped base of a bud by the growing stamens. There are no true petals in the flowers, the cap representing a fusion of these organs alone or with the sepals, although sometimes the mature structures are almost indistinguishable (Carr and Carr, 1968). Depending on the species, the stamens are brightly coloured white, yellow, pink or red, this colour giving the visual showy effect to *Eucalyptus* flowers (Figure 6.11).

Figure 6.11. ***Eucalyptus* flowers**

A. *E. robusta* flower showing displacement of cap (operculum)

B. *E. robusta* flower showing central green style and stigma and surrounding anthers

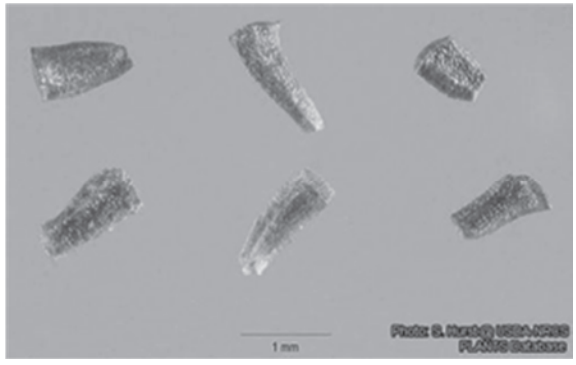
C. Bright pink stamens of *E.leucoxylon* flowers



Source: (A) and (B) courtesy Brian Johnston, 2007; (C) Jean Tosti, licenced under CC BY-SA 3.0.

After flowering and fertilisation, most species develop hard, woody seed capsules (fruits) (Figure 6.12). Each capsule usually contains fertile seeds alongside unfertilised ovules termed "chaff". Examination of the capsules from 21 species of *Eucalyptus* from south-western Australia showed that the largest seeds were 9 times wider than the smallest and 200 times heavier, while the number of fertile seeds per capsule varied from 1 to over a 100. These differences were somewhat associated with subgenera, species from *Monocalyptus* usually having fewer fertile seeds than *Symphyomyrtus* species (Gill, Brooker and Moore, 1992). Species that are subject to frequent fires produce smaller seeds, which are likely to provide superior abilities to germinate and colonise ash beds. Conversely, species that are less prone to fires produce larger seeds, the resulting larger seedlings being able to grow and establish over the periods between fires (Murray and Gill, 2001). Seeds are reported to be very small in the fast-growing plantation species (100 000-600 000 seeds per kg) (Eldridge et al., 1994).

Figure 6.12. **Seeds of *E. camaldulensis***



Source: Courtesy Steve Hurst, hosted by the USDA-NRCS Plants Database.

Nectar is produced from the base of the style, and attracts a wide range of insects, birds, possums and bats, which facilitate pollination. The few studies of nectar production

suggest that for *Eucalyptus* there is greater secretion at night, but at least one species of *Corymbia*, *C. gummifera*, appears to have no diurnal or nocturnal cycle (Goldingay, 2005; Horskins and Turner, 1999).

Development

Reproductive biology

Sexual reproduction

Eucalyptus species are usually capable of self-fertilisation, but in most cases breed by a combination of self-pollination and outcrossing, with the latter being more common. Outbreeding rates are relatively high (0.69-0.84; Moran and Bell, 1983) and are maintained by protandry.[6] Self-fertilisation frequently results in a reduction in the production of capsules, the number of seeds and in the vigour of the seedlings themselves (Eldridge et al., 1994; Potts and Wiltshire, 1997). However, although outcrossing may be favoured in *Eucalyptus*, its rate is dependent upon the number and density of trees, and even within a species, individual trees may be either fully self-fertile or almost incapable of self-fertilisation.

Extensive research supports the above conclusions. For example, in comparison to the analogous self-pollinations, cross-pollination of *E. nitens* resulted in greater numbers of healthy, developing ovules (Pound et al., 2003b) and cross-pollination of hybrids of *E. platypus* and *E. spathulata* resulted in more seeds per capsule (Wallwork and Sedgley, 2005). Experiments involving pollination of *E. grandis* with self and donor pollen demonstrated that the latter pollen results in the set of a greater number of seed, and in the case of pollinations with mixtures of self and donor pollen, the progeny were all the result of outcrossing (Horsley and Johnson, 2010). This is likely due to differential rates of pollen tube growth, a phenomenon which has been observed for self- versus donor-pollen tubes in *E. urophylla* and *E. grandis* (Horsley and Johnson, 2007). However, in other cases, the progeny of self-fertilisations may be selected against by mechanisms which operate after the formation of the zygote (James and Kennington, 1993). Regardless of the type of fertilisation, it is generally believed that the germination of pollen on the stigma is rapid, its viability on the stigma not exceeding a few days (Eldridge et al., 1994).

The reproductive success of any tree is linked to a range of factors. These include its age, location, health, and the number and size of its flowers. In *E. globulus* (Figure 6.13), the reproduction of individuals is associated with the size of flowers and features of the female reproductive organs, such as the size of the style and number of ovules (Suitor et al., 2009).

Asexual reproduction

Most eucalypt species can be artificially propagated by rooted cuttings provided the cuttings are taken from young seedlings (Eldridge et al., 1994). However, natural asexual means of reproduction such by the use of rhizomes and stolons, and the ability of tissues to give rise to small plantlets (natural vegetative propagation), is extremely rare. Only in isolated instances, amongst tropical woody species such as *E. porrecta*, *E. ptychocarpa*, and *E. jacobsiana*, as well as *E. moluccana* and *E. stellulata* which are found in more temperate climates, have rhizomes and/or stolons been observed (Gillison, Lacey and Bennett, 1980; Lacey, 1974).

Figure 6.13. ***Eucalyptus globulus***

Source: Rezerga, licenced under CC BY-SA 3.0.

Many species, especially the mallee eucalypts that are more tolerant to fire, drought and defoliation, can form lignotubers; these are woody swellings at the base of the stem from which a number of stems sprout forth to form multi-stemmed trees. Similarly, other species are capable of sprouting from epicormic buds (buds protected under the outer bark), following the destruction of their crowns by fire (Nicolle, 2006) (Figure 6.14). Some species are able to sprout from both lignotubers and stems (combination sprouters). Nutrient-rich structures such as lignotubers and sprouts from stems both promote the survival of plants in times of stress such as severe cold, and contribute to natural regeneration after fires. However, they do not contribute to the widespread dispersal of any species.

Regenerative strategies in the eucalypts have been collated by Nicolle (2006) and Rejmanek and Richardson (2011); based on these sources, information for the major plantation eucalypts is summarised in Table 6.3.

Pollen dispersal and pollination

The distribution of pollen from a source plant, whether by wind or animal, is usually described as being leptokurtic, being greatest a few metres from the source and then gradually decreasing with increasing distance (Levin, 1981). A pollinator will also likely carry pollen to a number of flowers. Although most will be deposited on the first few flowers, pollen can remain on the pollinator's body over extended visitations, leading to it being deposited on a flower long after it was collected.

Figure 6.14. **Shoots springing from *Eucalyptus* epicormic bud after bushfire**

Source: John O'Neill, Wikimedia, licensed under GNU FDL 1.2.

Table 6.3. **Regenerative strategies in Eucalyptus species**

Species	Lignotuber	Habit	Regenerative strategy	Source†
E. saligna	Variable	Tree	Lignotuber sprouter	s,c,f,r
E. grandis	Variable	Tree	Possible sprouter or obligate seeder	s,c,f,r
E. urophylla	Yes	Tree	Combination sprouter	s,c,f,r
E. pellita	Yes	Tree	Combination sprouter	s,c,f,r
E. tereticornis	Yes	Tree	Combination sprouter	s,c,f
E. camaldulensis	Variable	Tree	Sprouter (variable)	s,c,f,r
E. dunnii	Yes	Tree or facultative mallee	Combination sprouter	s,c,f,r
E. globulus	No	Tree	Combination sprouter	s,c,f,r
E. nitens	No	Tree	Stem sprouter	s,c,f,r

†Source of data: **s**: seedling examination (live and/or herbarium specimens); **c**: examination of late juvenile-stage individuals (saplings); **f**: field examination of mature individuals; and **r**: observation of response to fire in natural stands and/or cultivated individuals.

Source: Adapted from Appendix, Nicolle (2006) and Table 1, Rejmanek and Richardson (2011).

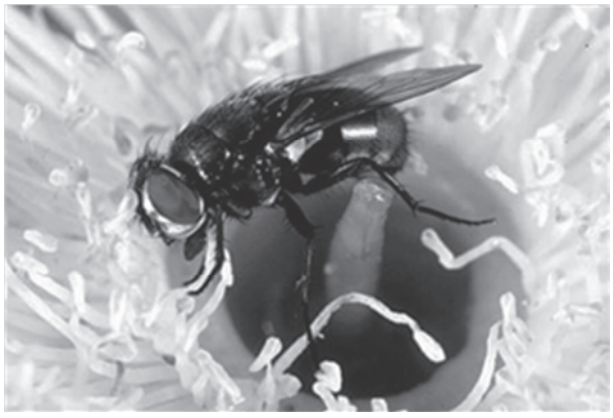
Eucalyptus species are mainly pollinated by vectors such as birds, insects and mammals, and reports of wind pollination are rare (House, 1997; Potts and Gore, 1995; Pryor, 1976). Wind pollination has been reported for *E. tereticornis* (Pryor, 1976) which has loose, non-sticky pollen, but this has not been verified (Potts and Gore, 1995). In Australia, it has been suggested that species with small flowers are predominantly pollinated by insects, while those with larger flowers are mainly pollinated by birds. However, the flowers of species are usually capable of being pollinated by all the above-listed vectors, a characteristic which may be of advantage in the often dry and unpredictable Australian climate (Ford, Paton and Forde, 1979). The major vector associated with any species is likely to be attracted by factors such as the type of nectar reward, the season and the weather at the time of flowering. For example, one localised study in Western Australia identified a number of birds as the major pollinators of *Eucalyptus* and other native Australian plants in winter (Hopper, 1981).

Nectar-feeding birds, of which honeyeaters are a representative, are a major feature of Australia and other southern hemisphere countries, but are extremely rare in the northern

hemisphere (Ford, Paton and Forde, 1979). As such, it is not surprising that in Australia the main birds that effect pollination of *Eucalyptus* are nectar-feeding ones. Honeyeaters, in particular, are likely the major pollinators of at least 200 species of *Eucalyptus*. Potentially, due to their visitations to greater numbers of flowers compared to insects, birds can lead to more cross-pollinations.

The principal insect pollinators of *Eucalyptus* are bees, flies and beetles. As with birds, their major interest in the flowers of this genus is the nectar. In Australia, both native bees and the introduced honey bee (*Apis mellifera*) act as pollinators, the latter being the major bee associated with the production of honey. Other insects, such as ants, butterflies and moths, probably play only a marginal role in pollination (Figure 6.15). Similarly, in Brazil, both *Apis mellifera* and other species of bees are probably the most important insect vectors of pollination (Barth, 2004; D'Apolito et al., 2010). Pollen can also be transferred between individuals, thus further enabling its dispersal.

Figure 6.15. **Pollinator (sheep blowfly) on eucalypt flower**



Source: CSIRO, licenced under CC BY 3.0.

Mammals that feed upon nectar and/or pollen are all potential vectors of pollen. In Australia, the most important are the arboreal marsupials, such as possums and gliders (gliding possums). For example, the yellow bellied glider (*Petaurus australis*) and the feathertail glider (*Acrobates pygmaeus*) are both known to feed on the nectar and pollen of *Eucalyptus*, likely inadvertently depositing pollen between flowers and trees (Goldingay, 1990; Turner, 1984). Fruit bats are also probably involved in pollination. The morphology of the tongues of some species is very similar to that of nectar-feeding birds and mammals, implying a diet high in nectar and the concurrent ability to pick up and transfer pollen (Birt, Hall and Smith, 1997).

In general, pollen dispersal in *Eucalyptus* is largely restricted to the immediate vicinity of its source, but patterns of pollen dispersal may change with spatial and temporal variation in the flowering resource and the pollinator community (Potts, Barbour and Hingston, 2001). For predominantly bird- (and flying fox-)pollinated eucalypts, pollen dispersal distance is likely to be greater than for those predominantly pollinated by insects.

Some cases of potential long-distance dispersal over several kilometres have been reported. For example, natural F_1 hybrids between *E. regnans* and *E. macrorhyncha* have been observed in forests of the latter, located over 5 km away from the closest stands of

E. regnans (Ashton and Sandiford, 1988). The occurrence of hybrids of *E. risodnii* in the range of *E. amygdalina* has suggested that, although pollen dispersal is largely confined to within a few metres of trees, it can occur several hundred metres from its source (Potts and Reid, 1988). Similarly, a study of pollen dispersal from *E. nitens* into a natural *E. ovata* population showed hybrid seed occurring at 200-300 m, though occasional hybrids were still found at 1.6 km from its source (Barbour, Potts and Vaillancourt, 2005). Examination of the trees of *Eucalyptus* plantations in Brazil by the use of isozyme electrophoresis has likewise demonstrated that pollen can travel several hundred metres across isolation belts of natural forest. In one study, it was demonstrated that 14.2% of seedlings in *E. grandis/E. urophylla* orchards showed evidence of crossing with plants 400 m distant (Campinhos et al., 1998), while in a second it was shown that pollen could travel over 800 m to fertilise plants (Junghans et al., 1998). In a seed orchard of *E. grandis* in Madagascar, 40% pollen immigration was observed over 100 m (Chaix et al., 2003). Long-distance pollen dispersal has also been observed for remnant populations of *E. wandoo*, where over 65% of pollen was found to be sourced from outside the populations at distances of at least 1 km (Byrne et al., 2008).

Fruit/seed development and dispersal

After pollination and fertilisation, the development of seed capsules usually takes several months before they are mature for harvest or release of seed. Species that originate in the northern regions of Australia and the islands to the north will usually shed their seeds after the capsules reach maturity. However, under natural circumstances, for species originating in temperate climates, the capsules invariably remain closed for a further 12-24 months, after which the valves open and release the seeds. Such trees are often characterised by a slow release of seeds throughout the year. In most cases, capsules release seed prior to any dissociation from a tree, but after exposure to fire and dropping to the ground they may soon open. Capsule abortion is associated with the level of fertilisation. Those capsules that have a low level of fertilised ovules are more likely to abort, but abortion is also influenced by the levels of nutrients available to the plant (Suitor et al., 2008). If seed capsules have been collected, placing them in a warm dry environment for several days will usually induce them to open and release seed. If seed is not to be sown, its viability is best retained by cold storage, seeds stored in this manner often remaining viable for at least 20 years.

Eucalyptus seeds are mainly dispersed by gravity and wind. Dispersal by animals (e.g. in fur of larger animals or intentionally by ants [House, 1997]) is unimportant, and dispersal by water dependent on either proximity to water courses or infrequent flooding. However, seed dispersal by water can be over long distances, and if this is seen as a problem, plants should not be grown near water courses (Rejmanek and Richardson, 2011). The distance of dispersal of seed from a tree is largely a function of the height of release, wind velocity and weight (Cremer, 1977). Generally, seeds have no adaptation for dispersal (wings or fleshy tissues), and wind or gravity will carry seeds no further from the base of the tree than the height of the tree (Cremer, 1977). Terminal velocities of seeds of all tested eucalypt species are between 2.0 and 5.5 metres per second. Although ants are considered the only invertebrate which can move plant seeds, they are not known to play a significant role in the dispersal of *Eucalyptus* seeds. However, in Australia, seeds of *E. torelliana* can be transported distances of over 300 m from their source by the bee *Trigona carbonaria*, which collects a resin to which the seeds adhere (Wallace and Trueman, 1995). Similarly, seeds of *Corymbia torelliana* are dispersed by related species of bees (Wallace and Lee, 2010).

The number of seeds produced is much lower than the number of flowers and ovules available for fertilisation. Possible reasons for this include the outlay of resources specifically to attract pollinators, production of excess pollen to increase the chances of fertilisation of the available ovules and the reduction of the impact of predators which target seed prior to dispersal (House, 1997). However, a model has been developed and successfully used for the estimation of the seed quantity for stands of *Eucalyptus*. This model factors in the number of branches in the crowns of trees, the number of capsules per branch and the average number of viable seeds per capsule (Bassett, White and Dacy, 2006).

Seed dormancy and germination

In general, eucalypt seeds do not display innate dormancy, i.e. inhibition of germination on a year-to-year scale (Gill, 1997). However, seeds may have an after-ripening period following dispersal, and short-lived dormancy may be induced by exposure to high temperatures (see Grose, 1960; Wellington, 1989). Seed storage in the soil is usually less than a year (Grose, 1960). Any substantial store of eucalypt seed is in the canopy of the plant: in temperate eucalypts such as *E. grandis*, seeds are retained in the woody capsules until hot, dry conditions or fire cause their release, while in forest trees and mallees there is a continuous, low level of seed release throughout the year (House, 1997). Therefore, if seedling establishment is to be successful in any eucalypt species, it is likely to take place within a year of seed dispersal (Gill, 1997).

Under both natural and artificial conditions, the germination of seed from *Eucalyptus* is dependent on a range of factors, including temperature, moisture, light and the mineral constituents of the soil. For example, germination of seeds of *E. globulus* in controlled environment chambers was found to be optimal at 28°C, and to be sensitive to water potential and the size of the seeds (Lopez et al., 2000). In the case of *E. delegatensis*, which grows in cooler temperate regions, the germination optimum was between 15°C and 20°C, and was found to be sensitive to both water potential and the soil matric potential (Battaglia, 1993). These, and other studies, underline a correlation between the optimal temperature for germination and the climate where *Eucalyptus* trees originate. Boland, Brooker and Turnbull (1980) have established temperature optima for germination of more than 400 species of *Eucalyptus*. South Australian species have germination optima between 15°C and 25°C whereas Northern Australian species have optima closer to 30°C. Further, comparison of seed-lots of one species taken from different areas can sometimes show significant differences in the rate of germination, underlying regional differences in populations (Humara et al., 2000).

The presence of moisture is an essential pre-requisite for successful germination and subsequent establishment of seedlings. In Australia, germination in the wild is usually linked to the season of rainfall in a particular region. Often, controlled seed germination can also be promoted by exposure to heat, cold, smoke, scarification and/or the use of specific light-darkness regimes (Bell, 1994; Close and Wilson, 2002). Plants native to regions with Mediterranean climates, such as Australia, are frequently dependent upon either heat or smoke from fires to help stimulate germination (Moreira et al., 2010; Read et al., 2000). Under controlled conditions, the germination of some species, such as *E. blakelyi*, is increased significantly by the use of light (Li et al., 2003), while that of other species, such as *E. globulus*, is enhanced by constant darkness, as opposed to a constant light or a mixed light-dark regime (Nair, Wilson and Spurr, 2009). Although cold may help seeds of some species germinate, there is likely to be an increase in the

mortality of germinating seed of most species exposed to surface frost (Cremer and Mucha, 1985).

Seed stratification, a technique that involves placing the seed in a moist environment for defined time periods, can also enhance germination (Donald and Jacobs, 1993), as can the use of osmotic solutions of polyethylene glycol, which are used to control water potential (Donald and Lundquist, 1988). Often, the consecutive use of more than one of these methods will lead to a greater level of seed germination.

In general, it appears that species from the subgenus *Symphyomyrtus* have a higher root-to-shoot ratio and can establish themselves faster than those from *Monocalyptus* (Davidson and Reid, 1980). However, the successful establishment of seedlings is largely dependent upon the surrounding environment. Usually the growth of seedlings is slow, and mortality high, in areas of established forest. In some circumstances this may be due to the release of allelopathic chemicals which result in a general suppression of understorey growth in *Eucalyptus* forests (May and Ash, 1990). Fires, which in natural circumstances have been part of the Australian environment and to which its flora is adapted, present opportunities for the establishment of new seedlings (Jurskis, 2005). For some species, such as *E. regnans*, the creation of both large forest gaps and destruction of understorey plants by fires may be essential for regeneration (Ashton and Chinner, 1999; Van der Meer and Dignan, 2007). From an industrial perspective, larger gaps would be expected to result in increased timber yields.

Genetics

As with most other plants of commercial significance, there have been recent rapid advances in research concentrating on the genetics and genomics of *Eucalyptus* species in particular, as well as other species in the Myrtaceae (Grattapaglia et al., 2012).

Although there are some reports of varying chromosome numbers among species of *Eucalyptus*, it is likely that all species are characterised by a diploid (2n) number of 22, corresponding to that observed among virtually all examined plants in the Myrtaceae family (Bachir and Abdellah, 2006; Rye, 1979). Reports of higher chromosome numbers in some species may be the result of the fragmentation of certain chromosomes while they were being prepared for counting. There are no reports of the occurrence of natural polyploids in the genus, but occasionally in counting chromosomes from individual plants, cells with a tetraploid number (4n = 44) are encountered.

In one study, the nuclear (2C) DNA content of 12 *Eucalyptus* species and 5 hybrids, including some of the species most widely grown around the world, was estimated to range from 0.77 pg/2C to 1.47 pg/2C, equivalent to haploid genome sizes of 370-700 megabase pairs (Grattapaglia and Bradshaw, 1994). A more recent investigation of the genomes of *E. globulus*, *E. grandis* and *E. urophylla* suggested that the sizes of their nuclear DNAs were between 1.05 pg/2C and 1.41 pg/2C, corresponding to approximately 500-600 megabase pairs per haploid genome (Praca, Carvalho and Novaes, 2009). For comparison, the nuclear DNA content of *Arabidopsis thaliana* (selected as a "model" plant species partly on the grounds of its small genome size) is 0.32 pg/2C, while those of banana, *Brassica rapa*, cotton and wheat are 1.26, 1.6, 4.8 and 34.66 pg/2C, respectively (Bennett and Leitch, 2012).

Maps of molecular markers have been constructed for a range of *Eucalyptus* species, including *E. grandis*, *E. globulus*, *E. nitens* and *E. urophylla*, as well as a number of commercial hybrids (Grattapglia et al., 2012). These maps represent AFLP, RAPD, RFLP

and SNP markers, most covering over 90% of the respective genomes. A DArT genome array has been developed that has approximately 1 000-2 000 polymorphic markers that can be used for population studies and linkage mapping in most *Eucalyptus* mapping populations (Sansaloni et al., 2010). The sequencing of the clones on the array will enable the integration of the sequence of any *Eucalyptus* genome with the location of QTLs and other markers. By using over 4 000 DArT and microsatellite markers, a high-density marker map for *Eucalyptus* has also been produced, the average interval between adjacent markers being 0.31 cM (Hudson et al., 2012a).

Recent studies have used next generation sequencing to produce *Eucalyptus* expressed sequence tags (ESTs) and develop single-nucleotide polymorphisms (SNPs). Using a pool of cDNAs from different tissues and genotypes of *E. grandis*, nearly 150 Mbp of expressed sequences could be assembled. Further, alignment of the sequences from the different genotypes allowed the detection of over 23 000 SNPs (Novaes et al., 2008). In another study, 23 genes from individuals of *E. globulus*, *E. nitens*, *E. camaldulensi*s and *E. loxophleba* were sequenced, identifying over 8 500 SNPs, with *E. camaldulensis* averaging one SNP every 16 bp for the sequenced genes (Kulheim et al., 2009). Lastly, by using a 1.2 million EST dataset, consisting of both Sanger and Next Generation sequences from six *Eucalyptus* sequences (representing three sections of the subgenus *Symphyomyrtus*), it was possible to develop a set of 768 genome-wide SNPs (Grattapglia et al., 2011). These were assayed in *Eucalyptus* using the Golden Gate genotyping technology, their reliability as SNPs being extremely high.

Rapid advances in genomics and the techniques of recombinant DNA technology have led to the characterisation and sequencing of the genomes of an ever-increasing number of plants. Data from such research can then be applied in programmes aimed at both understanding the fundamental developmental process of plants and genetically engineering plants with desired traits. The ~600 Mbp genome of *E. grandis* is being sequenced by the US Department of Energy, Joint Genome Institute (DOE-JGI). An assembly sequence is available.[7] The DOE-JGI has also conducted sequencing of a clone (X46) of *E. globulus*, while the Japanese Kazusa DNA Research Institute has produced a draft sequence for *E. camaldulensis*, showing that the genome is approximately 650 Mbp and consists of over 77 000 (complete or partial) genes (Hirakawa et al., 2011). To facilitate the map-based cloning of genes in *Eucalyptus*, BAC libraries have been constructed from the species *E. grandis* (Paiva et al., 2011).

Quantitative traits within *Eucalyptus* have been the subject of much research. Recent studies include those on cold hardiness and growth in *E. urophylla* x *E. tereticornis* hybrids (He et al., 2012), lignin composition and growth traits in *E. urophylla* (Mandrou et al., 2012), and resistance to the rust fungus *Puccinia psidii* in *Eucalyptus* species (Alves et al., 2012). The understanding and evaluation of QTLs is further enhanced by the use of genomic selection, a technique that may enable breeding times in *Eucalyptus* to be drastically reduced (Resende et al., 2012).

Collections of ESTs for several species, representing a number of organs and growth conditions, have been generated in Australia, Brazil, France and the United States (Teulieres and Marque, 2007). Some of these collections are publicly available while others belong to private companies. Published EST isolations include a collection of those preferentially expressed in the xylem tissue of *E. gunnii* (Paux et al., 2004) and from a cold acclimatised line of the same species (Keller et al., 2009). Bioinformatic (BLAST) searching of the Brazilian FOREST EST database has identified sequences from genes

that, in other species, are known to be involved in both abiotic and biotic stresses (Rosa et al., 2010).

Both chloroplasts and mitochondria are usually maternally inherited in angiosperms, and *Eucalyptus* appears to be no exception (Byrne, Moran and Tibbits, 1993; Vaillancourt, Petty and McKinnon, 2004). Not only does this form of inheritance occur in individual species, but at least in the case of chloroplast DNA, the barriers which prevent pollen mediated transmission appear to operate identically in hybrids between species (McKinnon et al., 2001). The chloroplast genomes of *E. globulus* and *E. grandis* have been sequenced, establishing that they share over 99% sequence identity, together with identical gene orders (Paiva et al., 2011; Steane, 2005). The genes found on these organelle genomes are not significantly different from those established for other angiosperms.

From the perspectives of ecology and silviculture, genetic variation within a *Eucalyptus* plantation species reduces the competition between individual plants and promotes coexistence with other species (Boyden, Binkley and Stape, 2008). However, where competition between individual trees is low (as may be the case when they are widely spaced), trees of identical genetic (clonal) origin are likely to outperform those with genetic diversity.

Abiotic interactions

As with other plants, abnormal growth in *Eucalyptus* is almost always a symptom of an abiotic or biotic stress. Abiotic stresses include nutrient deficiencies, metal toxicities, the effects of extremes of temperature, excess or deficiency in water, and even the presence of pollutants in the air.

Nutrient stress

The supply of nutrients is important not only to *Eucalyptus* trees in their native Australasian habitats, but in all areas around the world where *Eucalyptus* species are grown as plantation crops. Australia is renowned for its high proportion of nutrient poor soils, even when compared to the arid or semi-arid zone soils in other continents; in particular, many Australian soils are deficient in phosphorus (Orians and Milewski, 2007). However, although *Eucalyptus* species are thus adapted to growing in environments where nutrients may be deficient, they can nonetheless show distinct symptoms of stress when one nutrient (or more) becomes limiting.

Nutrient in plants can be broadly classified into three groups: those that are phloem mobile from leaves (nitrogen, phosphorus, potassium), those that are immobile from leaves (boron, calcium, iron, manganese) and those that are mobile only under only certain conditions (copper, magnesium, sulphur, zinc) (Dell et al., 2002). Indicative symptoms of stress are the colour and shape of leaves (e.g. chlorosis and/or necrosis of leaf tissue), the shape and the presence of leaves in the canopy, and the thickness of the stem compared to healthy plants (Snowdon, 2000).

Nitrogen and phosphorus feature prominently as essential elements, the former being part of nucleic acids and proteins while the latter occurs in nucleic acids and important cellular molecules such as ATP. The availability of phosphorus and nitrogen in soils is often linked, and a balanced supply of both nutrients is needed for ideal growth. Deficiency in nitrogen is frequently characterised by the yellowing of leaves in

Eucalyptus, while that of phosphorus by the formation of purple patches and necrosis on leaves (Dell et al., 2002).

Symptoms of deficiencies in other nutrients include the scorching of leaves, sickle-shaped leaves, impairment of the growth of the shoot tip and loosely hanging branches. Sometimes the visible signs of stress relating to the deficiency of one element are virtually identical to those of another, making it difficult to ascertain the cause of the problem. For example, uniformly yellow leaves on a plant may be indicative of either nitrogen or sulphur deficiency.

In soils where the level of nitrogen and phosphorus is so low that it is restricting the growth of trees, the use of fertilisers is common. The application of a nitrogen- and phosphorus-based fertiliser for three years to trees of *E. grandis* in Queensland, Australia, demonstrated that it significantly increased both tree heights and basal areas as compared to non-fertilised controls, the growth of the latter in fact being severely inhibited by a low supply of nutrients (Cromer et al., 1993a; 1993b). In the southern Australian state of Tasmania, where low levels of nitrogen in soils can limit the growth of *E. nitens*, the application of a nitrogen fertiliser was found to increase growth, the availability of nitrogen in treated soils remaining elevated for one to two years after treatment (Smethurst et al., 2004).

Other studies, in Argentina and China, have demonstrated that the application of phosphorus increases the growth of trees, and is able to concurrently elevate the extraction of nitrogen from soils, plants accumulating more nitrogen than those fertilised with nitrogen alone (Graciano et al., 2006; Xu et al., 2002). The application of fertilisers is common in the large plantations in Brazil, substantially increasing productivity, most often by accelerating the growth of trees (Goncalves et al., 2008).

Toxicity of metals

The discharge into the environment of metals, especially cadmium, chromium and aluminium, can be a problem in areas where industry is located. These metals can find their way into all biological organisms, affecting their health and ability to reproduce.

Approximately 30% of the world's soils are acidic, such soils being particularly common in the humid temperate and humid tropical regions of Australia, Asia, Africa, India, and Central and South America. These soils can restrict, if not inhibit, the growth of plants, in turn leading to the failure of crops and the impoverishment of people. Aluminium toxicity is often associated with acidic soils, reducing the growth of roots and their efficient uptake of water and nutrients (Eswaran, Reich and Beinroth, 1997). Growth of seedlings of six *Eucalyptus* species in liquid media with varying concentrations of aluminium showed that they had different degrees of tolerance to high aluminium levels, but the elongation of their roots was actually promoted by low levels (Silva et al., 2004).

It is possible to reduce the effects of aluminium toxicity by the addition of lime to soils, but often this is not economically or physically possible. In addition to the use of *Eucalyptus* cultivars that are inherently resistant to aluminium toxicity, it has also been reported that certain fungi can prove beneficial in such soils. Concurrent inoculation of both saprobe and arbuscular mycorrhizal fungi was observed to increase the resistance of *E. globulus* to aluminium (Arriagada et al., 2007), whereas inoculation with a mycorrhizal fungus was associated with lower aluminium accumulation in shoots of *E. tereticornis* (Khosla and Reddy, 2008). Plants can also respond to aluminium toxicity by the excretion of organic acids. In the case of *E. camaldulensis*, a number of membrane

proteins involved with the excretion of citrate have been isolated and characterised (Sawaki et al., 2013).

Cadmium is a by-product of the refining of zinc, being used in nickel/cadmium batteries and as a corrosion-resistant coating, while chromium figures prominently in the pigments of paints and other commodities. Both metals accumulate in organisms, have been demonstrated to be both toxic to *E. camaldulensis* (Shah et al., 2011) and, at least in the case of chromium, to inhibit the growth and colonisation of *E. urophylla* by the mycorrhizal fungus *Pisolithu*s (Aggangan, Dell and Malajczuk, 1998).

Temperature stress

Many species of *Eucalyptus* are sensitive to frost, with species of *Monocalyptus* being generally less resistant to frost than those of *Symphyomyrtus* (Noble, 1989). As might be expected, there is usually increased tolerance to frost in the species that grow at higher altitudes and/or are more often exposed to colder temperatures. Within a given species, individuals may demonstrate a range of responses to frost, indicating that the selection and breeding of populations with tolerance is possible (Doran et al., 2005). Further, continual exposure to low non-freezing temperatures, a process called hardening, can acclimatise plants to freezing temperatures, enabling them to resist frosts. This process may be accompanied by an elevation in the level of soluble sugars, and at least in the case of cell suspension cultures, incubation with soluble sugars increases the frost-hardiness of these cells (Travert et al., 1997).

At the other extreme, many *Eucalyptus* species native to the dry areas of Australia are well able to withstand high temperatures and the associated frequent drought conditions. However, seedlings are prone in particular to heat stress and mortality from high temperatures. To cope with heat, seedlings develop roots with deep and wide penetration, while it is not uncommon for adult plants to continually shed leaves during dry conditions to reduce the loss of water. At a fundamental level, there appears to be a direct relationship between the distribution of *Eucalyptus* species and their optimum temperature for growth and ability to withstand extreme conditions (Paton, 1980).

Water stress

The response of plants to drought is usually linked to changing the balance between an investment in the growth and maintenance of shoots (including the leaves), as opposed to that of roots. As shoots, and leaves in particular, are more prone to water loss than roots, it is ideal for a plant that is adapted to drought conditions to have a large root system, or if a plant is experiencing drought to expand its root system at the expense of the aerial organs. Often linked to this is the ability to control the loss of water through stomata. Any plant exposed to drought must also be able to maintain the efficient conduct of water through its xylem.

Eucalypts use extensive and deep root systems to access water and close their stomata for longer periods in the day to prevent loss of water (Costa e Silva et al., 2004; Eldridge et al., 1994). Osmotic adjustment as a means of enhancing turgor maintenance, and even the intercellular storage of water, are used by some species of *Eucalyptus* to cope with drought (Ladiges, 1974; Myers and Neales, 1986). As in the case of frost tolerance, the ability of *Eucalyptus* seedlings to withstand drought can be enhanced by a process of hardening. For example, seedlings of *E. pilularis* were drought hardened by reducing irrigation, and after transplantation to a glasshouse drought regime were seen to have increased survival (Thomas, 2009).

Conversely, exposure to excess water can drastically reduce the growth and development of plants. Waterlogged soils invariably restrict the uptake of oxygen by roots, in turn reducing aerobic respiration and inducing reliance upon anaerobic respiration, the latter being accompanied by the production of toxic organic and inorganic molecules. *Eucalyptus* species that grow waterlogged soils have evolved a number of adaptations, including adventitious roots, aerenchyma (pores to allow diffusion of oxygen from the shoot to root) and hypertrophy (swelling) of the stem. The ability of *E. camaldulensis* to withstand flooding has been attributed to its ability to produce ethylene, which results in hypertrophy (Blake and Reid, 1981; Van der Moezel et al., 1988). In one study of the three *Eucalyptus* species – *E. grandis*, *E. robusta* and *E. saligna* – the first was found to be the most resistant to waterlogging, this phenotype being dependent upon the formation of adventitious roots (Clemens, Kirk and Mills, 1978).

Salinity stress

Many areas of Australia, both along both the coast and inland, including those with a tendency for waterlogging, are characterised by saline soils. Some species such as *E. camaldulensis*, *E. tereticornis* and *E. occidentalis*, all in the subgenus *Symphyomyrtus*, have a high resistance to saline conditions, while species in the subgenus *Monocalyptus* are frequently salt sensitive (Benyon et al., 1999; Marcar, 1989; Sands, 1981). Increased salinity is often associated with reduced tree growth, this being manifested in decreased stem diameter and crown volume. Stomatal conductance and photosynthetic rates decrease under saline conditions; during summer, when water stress is more likely, the concentrations of salt in the leaves increases (Barrett, Preiss and Sinclair, 2005; Van der Moezel, Watson and Bell, 1989).

There are believed to be two general mechanisms of salinity tolerance in plants: 1) the ability of the plant to keep salt ions away from cells and/or tissues where they would be particularly harmful; and 2) the ability of a tissue to tolerate the elevated level of salt ions. In the former case, either active or passive methods are used to exclude and extrude ions, while in the latter the focus is on compartmentalising ions in cellular organelles such as vacuoles. It has been proposed that the relative tolerance of *E. camaldulensis* to salt is possibly linked to plant tissues being able to tolerate the ion, but in other *Eucalyptus* species, tolerance may be associated with reduced uptake of ions from the surrounding environment (Sands, 1981). Stomatal closure and a reduction in stomatal conductance is a feature of the response of *E. camaldulensis* and *E. lesouefii* to high salinity (Van der Moezel, Watson and Bell, 1989).

Air pollution and global warming

Gaseous pollutants arising from industry can affect the growth and survival of all living organisms. In many countries, forests nearby to industrial centres show obvious signs of damage from air pollutants. In China, sulphur dioxide and fluoride in the air have been linked to foliar damage in many trees, including *Eucalyptus* species adjacent to large cities (Shu-Wen et al., 1990), while pollution from cars can reduce the levels of photosynthetic pigments in *Eucalyptus* (Joshi and Swami, 2009). Other research has demonstrated that ozone (O_3), which is a product of fuel combustion, can significantly reduce the weight and injure the leaves of certain species of *Eucalyptus* (Monk and Murray, 1995). *Eucalyptus* trees exposed to chemicals in the air respond by the activation of enzymes such as peroxidases, ascorbate peroxidases and catalases, as well as by

increasing the cellular levels of the antioxidant ascorbic acid, all of which help provide protection (Seyyednejad and Koochak, 2010).

The possible effects of global warming on plants have been the subject of much research as well as controversy. An increase in the levels of carbon dioxide may prove beneficial to certain species of *Eucalyptus*, providing they have an adequate supply of water (Ghannoum et al., 2010), but the possibility of increased droughts may negate this advantage. In Australia, it is likely that any reduction in rainfall will increase tree mortality, the frequency and intensity of bushfires, and change the nature of pest and pathogen risks (Booth, Kirschbaum and Battaglia, 2010). One study of the effect of global warming on plantations of *Eucalyptus* in the Brazilian states of Espirito Santo and Bahia has suggested that the yield from these forests could decrease by at least 24% by the end of this century due to an increase in the severity and duration of droughts (Baesso, Ribeiro and Silva, 2010).

Biotic interactions

In their native environments in Australia, Papua New Guinea, Indonesia and the Philippines, as well as in many other countries to which they have been introduced, *Eucalyptus* species interact with mycorrhizal fungi and are affected by a wide range of diseases and animal pests. In particular, the effective management of pests and pathogens in plantations is a necessary prerequisite for their commercial success. As an exotic species, *Eucalyptus* trees are susceptible to elements of the biota of their new homes as well as known Australian pests that have been accidentally introduced. Nevertheless, there are many more pests and pathogens of *Eucalyptus* in their native habitats than occur outside their native range.

Mycorrhizas

Mycorrhizal fungi form symbiotic relationships with the roots of most land plants, and this interaction needs to be studied to fully understand plant-soil relations (Rosendahl, 2008). Both arbuscular mycorrhizal fungi and ectomycorrhizal fungi are known to colonise the roots of *Eucalyptus*, individual fungal species having preferences for particular plant species (Pagano and Scotti, 2008). These fungi help provide plants with necessary minerals (such as nitrogen and phosphorus), while some protect plants from pathogenic fungi and toxic compounds, in return receiving carbohydrates from plants (van der Heijden, Bardgett and van Straalen, 2008). In nutrient-rich environments, the mycorrhiza may have little obvious effect on the plant, but in mineral-deficient environments the presence of the fungi may be essential for optimal growth and development (Schmidt, Handley and Sangtiean, 2006). Further, at least in some cases, mycorrhiza can protect *Eucalyptus* trees from the effects of elevated aluminium in the soil (Arriagada et al., 2007).

Species of these fungi have been introduced from Australia to other countries, almost certainly with the seedlings of the introduced plants (Vellinga, Wolfe and Pringle, 2009). Although it is not common for these fungi to form mycorrhizas with the local tree species, in these new environments some have spread from the native hosts to other plant species. For example, in the Iberian Peninsula, the ectomycorrhizal fungus *Laccaria fraternal* has spread from plantations of introduced *Eucalyptus* trees to a native *Cistus* sp. (Diez, 2005), while species of *Pilothus* have been introduced to many countries, including Brazil (Kasuya et al., 2010). On the other hand, in countries where it is an exotic,

Eucalyptus species have been colonised by members of the native mycorrhizal population.

Pathogens and diseases

Eucalypts in native forests in Australia have a wide range of co-evolved pathogens, mainly foliar pathogens in the Phylum *Ascomycota* (Park et al., 2000). These have only come to prominence with their increased incidence and severity in eucalypt plantations planted in the region. Perhaps the most important example is *Mycosphaerella* (*Teratosphaeria*) *cryptica*, causing serious leaf blight and defoliation in *E. globulus* plantations in southern Australia. Another highly destructive disease in native forests in southern Australia has been eucalypt dieback, caused by the oomycete *Phytophthora cinnamomi*, which is thought to have been introduced to the southern forests from tropical regions (Shearer and Smith, 2000). This pathogen has been particularly destructive in the jarrah (*Eucalyptus marginate*) forests of southern Western Australia.

Eucalypt species planted outside Australia are susceptible to a range of bacterial (including phytoplasma), fungal and viral diseases, many of which are caused by new-encounter pathogens. The most important of these are vascular wilts, mildews, leaf spots and blights, stem rots and cankers, and root rots. Especially amongst the fungi, pathogens of *Eucalyptus* come from a large number of different taxonomic groups, including *Basidiomycota*, *Cryptosporiopsis*, *Erysiphe*, *Erythricium*, *Mycosphaerella*, *Phaeophleospora* and *Sphaerotheca*. Other than natural host resistance, factors such as climate and environment are principally responsible for determining the severity of disease caused by these organisms.

The response of any exotic species to exposure to new diseases is unpredictable in the absence of a long period of co-existence and co-evolution. Resistance by any plant species is often, but not always, dependent upon natural selection acting on the genetically variable population of individuals exposed to the pathogen, resistant individuals more often surviving infection and breeding. In this context, introduced *Eucalyptus* trees have undoubtedly been resistant to many local pathogens, but succumbed to others.

The characterisation of host shifts of pathogens to *Eucalyptus* is of such importance that not only have the identified examples been the subjects of study, but much research has been conducted into the potential occurrence of such shifts. The destructive epidemics of *Phytophthora cinnamomi* in southern Australia appear to be a classic example of a new-encounter disease (Keane et al., 2000). Several fungal pathogens present on certain Myrtaceae species native to South America and South Africa have been considered capable of infecting introduced *Eucalyptus* (Pavlic et al., 2007; Perez et al., 2010). In the reverse direction, the movement of pathogens accompanying introduced *Eucalyptus* plantation trees to other plants, especially those in the family Myrtaceae, is important (Perez et al., 2008). Even in the Australian context itself, *Eucalyptus* faces new pathogens when grown outside their native range. For example, *Botryosphaeria australis* has been found infecting plantations of introduced *E. globulus* in Western Australia, the fungus almost certainly coming from adjacent forest trees and representing an extension of its host range (Burgess, Sakalidis and Hardy, 2006).

The major pathogen-induced diseases affecting *Eucalyptus* species worldwide are listed in Table 6.4.

Ralstonia solanacearum, a phytopathogenic soil bacterium, is the causal agent of bacterial wilt. It colonises the xylem, usually causing decolouration of this tissue and wilting of either individual branches or the entire crown of plants, the end result often being the death of the plant (Old, Wingfield and Yuan, 2003). This pathogen has a wide host range, infecting over 200 plant species from over 50 plant families. Reports of it infecting eucalypts in plantation have come from Australia, Brazil, China, Indonesia, Chinese Taipei, Thailand, Bolivarian Republic of Venezuela, Viet Nam and a number of sub-Saharan African countries (Fouche-Weich et al., 2006; Old, Wingfield and Yuan, 2003). There is no effective control measure. Although the culling of infected trees is possible, the bacteria survive in the soil and any remaining roots. This disease is rarely seen in the native forests of Australia, but has become evident as a problem in plantations.

Table 6.4. **Major pathogens affecting commercial *Eucalyptus* species in plantations**

Causal organism	Country of occurrence	Damage to plant	Reference
Ralstonia solanacearum (race1 and either biovar. 1 or 3) (Bacterium)	Australia, Brazil, China, Democratic Republic of the Congo, Indonesia, South Africa, Chinese Taipei, Thailand, Uganda, Venezuela, Viet Nam	Infection of xylem causing wilting (vascular wilt)	Old, Wingfield and Yuan (2003)
Botryosphaeria spp. (Ascomycota)	Worldwide	Stem cankers which may girdle the stem	Old, Wingfield and Yuan (2003); Pavlic et al. (2007)
Chrysoporthe cubensis (Ascomycota)	Africa, Caribbean (Cuba, Puerto Rico), Mexico, South America, South East Asia (Indonesia, Thailand, Viet Nam), United States	Cankers at the base of plants, but may extend up the stem	Rodas et al. (2005a)
Coniothyrium zuluense (anamorph of Ascomycota)	Argentina, Ethiopia, Hawaii, Mexico, South Africa, Thailand, Uganda, Uruguay, Viet Nam	Cankers in young green tissue	van Zyl, Coutinho and Wingfield (2002); Wingfield, Crous and Coutinho (1996)
Cylindrocladium spp. (anamorph of Ascomycota)	Australia, Colombia, India, South East Asia	Foliar and shoot blights, leaf spots, root lesions	Blum and Dianese (1993)
Erythricium (Corticium) salmonicolor (Basidiomycota)	Brazil, Costa Rica, India, Indonesia, Philippines, South Africa, Viet Nam, Zambia	Pink-coloured pustules on branches and stems ("pink disease")	Seth et al. (1978)
Mycosphaerella spp.; *Teratosphaeria* spp. (particularly *T. cryptica*) (Ascomycota)	Worldwide (particularly Australia, Brazil, New Zealand, South Africa)	Leaf blotches, defoliation, shoot die-back	Crous et al. (2006); Hunter et al. (2011)
Puccinia psidii (guava rust or myrtle rust) (Basidiomycota)	North and South America (recently found in Australia)	Yellow or brown pustules on leaves, stems or fruits	Coutinho et al. (1998)

Coniothyrium stem canker caused by the fungus *Coniothyrium zuluense* is a serious disease of *Eucalyptus* species in plantations outside Australia (van Zyl, Coutinho and Wingfield, 2002). It was first reported in South Africa, but since has been found in a number of other countries, both in Africa, South East Asia, Hawaii and South America (Cortinas et al., 2004; Gezahgne et al., 2005). The earliest indication of infection is usually lesions on young green tissue, which then coalesce to produce large cankers. A plant suffering from an advanced infection will have cankers along its entire stem, leading to malformation of the stem, and often death of the tops of branches. At present, the only effective management strategy is the selection and breeding of resistant lines for release into plantations.

Chrysoporthe cubensis (formerly *Cryphonectria cubensis*) is a fungal pathogen of *Eucalyptus* species in all continents, although particularly in South America where it may have originated (Rodas et al., 2005a). It is relatively rare in Australia and is especially

rare in native forests (Pegg et al., 2010). The cankers caused by this pathogen are usually located at the base of trees, although they may sometimes occur higher up in branches. Once a tree has been girdled, it may wilt and in severe cases die. In Brazil, the selection for resistant lines of *E. grandis* x *E. urophylla* hybrids has proven a successful strategy in dealing with this disease. This pathogen has also been recorded outside the Myrtaceae (Wingfield et al., 2008).

Species of *Cylindrocladium* infect a wide range of plants including *Eucalyptus*, particularly affecting plantations in tropical and sub-tropical regions of India, South East Asia and South America (Blum and Dianese, 1993; Rodas et al., 2005b). Plants infected by these fungi exhibit a wide range of symptoms, including foliar and shoot blights, leaf spots, root lesions and cankers which girdle stems. In nurseries, fungicides have proven effective in controlling this pathogen, but along with management by chemical means, the selection of resistant clones has been actively pursued.

Puccinia psidii (guava rust) is the only rust fungus that has been well documented to infect eucalypts (Coutinho et al., 1998; Glen et al., 2007; Langrell, Glen and Alfenas, 2008). It is a typical new-encounter pathogen, having transferred to eucalypts growing in extensive plantations in Brazil from its native myrtaceous host guava (*Psidium guajava*). Although a native of South America, it now occurs in Central America, the Caribbean, and the states of California, Florida and Hawaii in the United States (Loope, 2010), infecting species belonging to a number of Myrtaceae genera. Usually it infects young tissues, causing the deformation and often death of leaves and flower buds. In severe cases, it stunts the growth of trees and sometimes even leads to their death, but most young infected trees show few symptoms as they grow older. Furthermore, infection is sporadic, being at least partly dependent upon climatic variables. Control of the rust is possible by the use of fungicides, but in Brazil resistant lines of both *E. grandis* and the hybrid *E. grandis* x *E. urophylla* have been selected and used in plantations. The ability of *P. psidii* to find new hosts in *Eucalyptus* and other Myrtaceae is of particular importance to Australia (Coutinho et al., 1998). Nearly half of the classified genera of Myrtaceae occur there, and most of the native species belong to that family. Indeed, a form of *P. psidii*, designated by the common name "myrtle rust", has been recently found infecting a wide range of native myrtaceous hosts, including *Eucalyptus*, in Australia (Carnegie et al., 2010). Its discovery in Australia in 2010, clearly linked to a breakdown in quarantine restrictions in the nursery industry, represents the first time any member of the *P. psidii* group has been found on that continent. Although its host range consists of many species in the family Myrtaceae, it has not been recorded commonly on *Eucalyptus*. Nevertheless, the common occurrence of the fungus on eucalypts in Brazil makes it a serious threat to eucalypts in Australia. Management of this rust in nurseries is dependent upon the destruction of infected plants and the application of fungicides, although these measures are of limited usefulness once the fungus has spread to native vegetation. The full impact of this recent pathogen introduction to Australia is yet to be determined.

Another example of a likely host shift which may prove to be significant in the future is the infection of *Eucalyptus* trees in Argentina and Uruguay with the bacterium *Erwinia psidii*, also a well-known pathogen of guava (Coutinho et al., 2011). Infection of *Eucalyptus* trees is characterised by the die-back of branches and stems, symptoms similar to those observed after infection of guava.

Although virus or virus-like diseases have been reported in *Eucalyptus*, there has been little research in this field. Infection by viruses appears to result in only limited symptoms, which may be transitory and disappear as the trees get older. At present, virus

diseases make a negligible impact on the commercial cultivation of *Eucalyptus* (Randles, 2010; Wardlow, Kile and Dianese, 2000).

Pests

In Australia, there are a large number of bird species that feed on either *Eucalyptus* nectar or the insects that commonly inhabit these plants (Landsberg and Cork, 1997). However, there are few species that feed on fruits and no known leaf-eating species. Insectivorous birds must play a role in controlling the number of insects, but the extent to which this occurs is still debated, especially in relation to years where there is an abnormal increase in insect numbers. It should also be appreciated that in Australia, the level of native vegetation in an area is proportional to the level of bird species (Ford, 2011). In Brazil, which has extensive *Eucalyptus* plantations with varying degrees of intensity of understory clearing, the diversity of bird species is likely to be dependent upon the richness of the understory, but none have been identified as major pests of eucalypts (Marsden, Whiffen and Galetti, 2001).

The major mammalian herbivores of *Eucalyptus* in Australia are possums, gliders, koalas, kangaroos and wallabies (Dungey and Potts, 2002; Landsberg and Cork, 1997). Possums can eat leaves, nectar, gums and fruit, while koalas almost exclusively target leaves, and even then they prefer the leaves of only a minority of all the *Eucalyptus* species. Kangaroos, and especially the smaller wallabies that occur more in the forested areas, can be important grazers of young plants in plantations.

As with other plants, insects target a wide variety of tissues in *Eucalyptus*; damage ranges from mild to severe and may even result in tree death. Numerous native Australian insect species have been recorded on *Eucalyptus*, both in the Australasian region and in other countries where these plants and their co-evolved insect herbivores have been introduced. Such insects include defoliators, leaf chewers, stem borers (affecting both bark and wood), sap suckers, and others which target seeds, pollen and nectar. Outside the Australian region, the insect pests include some originating in Australia itself and many local indigenous species. For example, in China, approximately 300 species of insect, mainly native to China, have been identified on *Eucalyptus*. Among these, about 30 cause severe damage and a further 60 moderate damage (Pang, 2003), while one study in India found more than 60 species on trees of this genus (Sen-Sarma and Thakur, 1983). In New Zealand, there are now over 50 species of insects which feed on *Eucalyptus*, of which approximately one-half specialise in plants of this genus (Withers, 2001).

The ability of an insect to move hosts appears to depend upon factors such as the physical structure of the leaf and the chemical nature of the plant cells, and pests usually choose plants of similar chemistry and/or taxonomic relatedness to those to which they are adapted (Becerra, 1997). In this context, it should be noted that Australia, southern Africa and South America have a greater diversity of Myrtaceae plants than is found anywhere in the northern hemisphere (Paine, Steinbauer and Lawson, 2010). *Eucalyptus* trees in Brazil, for example, are often grown with internal strips of native vegetation, which may act as sources of insects that may be able to switch hosts. However, *Eucalyptus* has also attracted pests with no known preference for plants from the Myrtaceae.

Eucalyptus plantations have sometimes become a "refuge" for insect pests indigenous to the new countries in which they are planted, this being due to predators of these pests preferring not to live in the plantations (Grosman et al., 2005). The problem of insect pests has been tackled by a number of different measures, including chemical sprays and

the implementation of management practices based on biological control. In a number of cases, the introduction of a parasitoid insect has been effective in reducing the numbers of *Eucalyptus* insect pests (Dahlsten et al., 1998; Hanks, Paine and Millar, 1996; Luhring et al., 2000). Table 6.5 summarises the major insect pests of *Eucalyptus*. Some of these are discussed in more detail below.

Table 6.5. **Major insect pests affecting commercial *Eucalyptus* species**

Causal organism	Country of occurrence	Damage to plant	Reference
Ctenarytaina spp. (particularly *C. eucalypti*)	Australia, France, Italy, New Zealand, Portugal, South America, Spain, United States, Uruguay	Sucks the sap from trees	Gill (1998); Queiroz Santana and Burckhardt (2007)
Eriococcus coriaceus	Australia, New Zealand	Sucks the sap from trees	Vranjic and Gullan (1990)
Eupseudosoma involuta and *E. aberrans*	Brazil	Defoliation by caterpillar (larva)	Zanuncio et al. (1994)
Gonipterus gibberus, *G. scutellatus*	Argentina, Australia, Brazil, France, Italy, Kenya, Madagascar, Malawi, Mauritius, Mozambique, New Zealand, Uganda, United States, Uruguay, Zimbabwe	Damage to edges of leaves, defoliation, stunting and possible death of trees	Clarke, Paterson and Pennington (1998)
Isoptera (termites)	Worldwide	Bark and wood	Constantino and de Almeida Pessoa (2010); Landsberg and Cork (1997)
Phoracantha semipunctata and *P. recurva*	Argentina, Australia, Brazil, Chile, Cyprus*, Israel, New Zealand, South Africa, United States, Zimbabwe	Bark and cambium, possible girdling of trees and death	Paine and Millar (2002)
Sarsina violascens	Argentina, Brazil	Defoliation by caterpillar (larva)	Zanuncio et al. (1994)
Stenalcidia grosica	Brazil	Defoliation by caterpillar (larva)	Pereira et al. (2001)
Thyrinteina arnobia	Brazil	Defoliation by caterpillar (larva)	Batista-Pereira et al. (2006)

**Note by Turkey:* The information in this document with reference to "Cyprus" relates to the southern part of the Island. There is no single authority representing both Turkish and Greek Cypriot people on the Island. Turkey recognises the Turkish Republic of Northern Cyprus (TRNC). Until a lasting and equitable solution is found within the context of the United Nations, Turkey shall preserve its position concerning the "Cyprus issue".

**Note by all the European Union Member States of the OECD and the European Union:* The Republic of Cyprus is recognised by all members of the United Nations with the exception of Turkey. The information in this document relates to the area under the effective control of the Government of the Republic of Cyprus.

A number of psyllids have been introduced from Australia into the Americas and Europe. In California, both the blue gum psyllid (*Ctenarytaina eucalypti*) and the red gum lerp psyllid (*Glycaspis brimblecombei*) have proven to be significant pests, damaging or killing thousands of *Eucalyptus* trees. However the use of parasitoid wasps such as *Psyllaephagus pilosus* and *P. bliteus* has helped limit the problem (Dahlsten et al., 2005; 1998).

Larvae and adults of the weevils *Gonipterus gibberus* and *G. scutellatus* (Eucalyptus snout beetle) feed mainly on the edges of *Eucalyptus* leaves, often leading to the defoliation and death of young plants. Originating in Australia, they have spread to most countries that grow *Eucalyptu*s plantations (Clark, Paterson and Pennington, 1998). At least in some countries, effective management of these pests has been achieved by the use of the parasitoid wasp *Anaphes nitens*, the larvae of which eat the eggs of *Gonipterus scutellatus* (Huber and Prinsloo, 1990).

Phoracantha recurva and *P. semipunctata* (Eucalyptus longhorned borers) are beetle pests of *Eucalyptus* that are native to Australia and have spread around the world (Luhring et al., 2000; Paine and Millar, 2002; Paine et al., 2000). Their larvae tunnel into the bark and cambium of trees causing extensive damage, frequently girdling trees and leading to their death. Use of natural enemies, such as the parasitic wasp *Avetianella longoi*, which lays its eggs within the eggs of these beetles, has proven to be an effective method of biological control (Hanks, Paine and Millar, 1996).

Termites (*Isoptera*) are another prominent pest of *Eucalyptus*, although infestation by these insects frequently does not result in the death of trees. In the Australian environment, termites often only attack *Eucalyptus* after fire or other damage to the tree, their activity being most common in tropical climates (Landsberg and Cork, 1997). Regarding other continents, native termites and the larvae of certain beetles (*Lepidiota stigma*, *Anomala* spp.) are prominent pests of *Eucalyptus* in Brazil, China and southern Africa, although their prominence as a pest is dependent upon regional factors (Calderon and Constantino, 2007; Constantino and de Almeida Pessoa, 2010; Pang, 2003). In China, termites usually infect the tap roots of seedlings and frequently lead to their death (Pang, 2003).

Other examples in countries of host shifts of native insect pests onto *Eucalyptus* include species of ants in Brazil, the lepidopterans *Sarsina violascens*, *Stenalcidia grosica* and *Thyrinteina arnobia* in Brazil (Paine, Steinbauer and Lawson, 2010) and the lepidopteran *Coryphodema tristis* in South Africa (Gebeyehu, Hurley and Wingfield, 2005). In Brazil, the moth *T. arnobia* is known to attack a number of native species within the Myrtaceae, including guava and jaboticaba, but since the introduction of *Eucalyptus* to that country, it has extended its host range to become a frequent pest of trees from this genus (Batista-Pereira et al., 2006; Grosman et al., 2005). The wood boring moth *C. tristis*, which in South Africa has long been known as a pest of native and introduced trees, has recently been found to be capable of damaging trees of *E. nitens* (Gebeyehu, Hurley and Wingfield, 2005).

Although examples are known of parasitic nematodes which infect *Eucalyptus*, none are important pathogens of these plants (Wardlow, Kile and Dianese, 2000). Studies in a number of countries have indicated that nematodes can indeed cause mortality of plants, but their effect on the commercial success of plantations is minimal.

Additional interactions

Eucalyptus oils have been shown to be effective pesticides and repellents, acting against a range of bacteria, fungi, nematodes and arthropods (Batish et al., 2008). Further, due to the environmental problems of synthetic chemical-based approaches, the use of natural product-based pesticides and repellents has become more attractive. In the case of *Eucalyptus*, the oils are easily extractable from leaves, and their chemical diversity provides a variety of candidates that can be screened for their effectiveness against given targets.

For example, the oils from a number of *Eucalyptus* species, in particular *E. dunnii*, have distinct insecticidal and repellent properties against *Sitophilus zeamais*, a species of weevil that is commonly found in maize (Mossi et al., 2011). Oils from the three species *E. staigeriana*, *E citriodora* and *E. globulus* all act as insecticides of the egg, larval and adult phases of the sand fly (*Lutzomyia longipalpis*) (Maciel et al., 2010). The most effective oil is that of *E. staigeriana*, which consists primarily of (+)-limonene, Z-citral and E-citral. The major constituents of the oils of *E. citriodora* and *E. globulus* are

β-citronellal and 1,8-cineole, respectively. In certain situations 1,8-cineole can be used as an insecticide against mosquitoes (Klocke, Darlington and Balandrin, 1987), but it has only moderate toxicity against flies (Sunkontason et al., 2004). The oils from *E. camaldulensis* and *E. urophylla*, in particular the chemical constituent α-terpinene, have been recorded as larvicides against some species of mosquito (Cheng et al., 2009). Anti-microbial properties include action against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Candida albicans* (Hendry et al., 2009; Marzoug et al., 2011).

Eucalyptus oils have also been demonstrated to have herbicidal properties. The oil from *E. citriodora* inhibits the germination and growth of *Bidens pilosa*, *Amaranthus viridis*, *Rumex nepalensis* and *Leucaena leucocephala*, all of which are weeds in India (Batish et al., 2004; Setia et al., 2007). The findings from such research may prove the basis of the development of bioherbicides. Although the oil from *Eucalyptus* itself can produce allergic contact dermatitis, it can be an effective insecticide against house dust mites, reducing the allergens associated with these insects in children's soft toys (Chang et al., 2011).

Weediness

Weediness status on a global scale

A weed can be defined as a plant that causes significant levels of one or more harms in a given geographical area. The most important of these harms are: 1) adverse effects on the health of people and/or animals; 2) reduction in the establishment and/or yield of desired plants; 3) restriction in the physical movement of people, animals or vehicles; and 4) adverse effects on environmental health, such as adverse changes to soil, salinity and the habitat of desirable organisms. Potential adverse effects on the environment from *Eucalyptus* include possible negative impacts on biodiversity, water quantity and quality, and fire risk.

Standard agricultural practices reduce biological diversity when compared to previous more natural ecosystems, and a eucalypt monoculture is not expected to be greatly different in this respect. It is unsurprising, then, that Stewart (2011) found that water quality and biodiversity values at a number of *E. globulus* plantation sites were inferior to those at remnant native vegetation sites. However, when compared with "pasture unfenced" and some "pasture fenced" sites", the plantation sites often had better water quality, riparian condition and biodiversity (Stewart, 2011). Studies in Brazil have shown that both *Eucalyptus* plantations and areas that were once planted with *Eucalyptus* trees but have been allowed to regenerate forest naturally, have fewer species of birds, ferns, epiphytic angiosperms and other organisms than observed in natural forests (Barlow et al., 2007; Fonseca et al., 2009). However, at least in some cases, plantations of *Eucalyptus* provide a unique habitat that is attractive to some species of birds with lifestyles that can benefit from the monocultures (Loyn et al., 2007).

Eucalypts are widely used in agroforestry and can provide environmental benefits for degraded landscapes. However, broad areas of revegetation may provide a large source of foreign genes in landscapes where small remnant native populations act as a sink (Byrne and Stone, 2011). Genetic change from hybridisation can threaten persistence of such populations through genetic assimilation or demographic swamping. The potential for gene flow and natural hybridisation of *Eucalyptus* is considered later in this chapter.

Eucalyptus trees are also known to be able to suppress the growth of understorey plants and adjoining crop plants, through the release of allelopathic chemicals (Zhang et al., 2010).

As outlined above, eucalypts may use extensive and deep root systems to access water. As a consequence, eucalypt plantations have been the subject of concerns about their potential for high water use and possible effects on groundwater (Almeida et al., 2010a; Morris et al., 2004). A number of authors have examined the impacts of such plantations on the environment, concluding that although they have adverse effects, if well managed they can provide benefits such as acting as wind breaks, reducing wind erosion and providing shelter for humans and animals (Poore and Fries, 1985). Nonetheless, there is data indicating that afforestation with *Eucalyptus* species can affect stream flow (Scott and Prinsloo, 2008; Silveira and Alonso, 2009); the degree to which this occurs has been found to depend on rainfall intensity and distribution, soil texture, tree age and stocking (Almeida et al., 2007).

Detailed data on water use and water balance of plantations are required to evaluate their environmental impact and to design optimal land-use strategies in catchment areas where wood production is an important economic component. Hydrology research for eucalypt and other exotic tree plantations has therefore received increasing attention in recent years. In Brazil, for example, studies of evapotranspiration and catchment water balance in eucalypt plantations have found that, in some parts of the country, catchment-scale plantation evapotranspiration did not differ from the climatic mean, whilst under other conditions it was higher (Lima et al., 2012a; 2012b). These authors concluded that, in general, there is no reason to expect that forest plantations are inherently detrimental to water availability, or that they would produce hydrological effects of the same magnitude in all situations. Instead, these and other studies' results show that the control of water impacts is very much dependent on the implementation of sustainable strategies of forest plantation management practices based on practical local experience and incorporating results from experimental studies and monitoring programmes (Lima et al., 2012a; 2012b). Selection of clones for water-use efficiency can also play a part in such strategies (Dye, 2012). Comparisons of water loss between *Eucalyptus* and pine plantations have also been conducted, with at least some work suggesting that plantations of the latter have less effect on stream flow (Dvorak, 2012).

Characteristics of weeds may be related to potential invasiveness, such as high seed output, rapid growth to flowering, self-fertilisation and secondary seed dormancy. Further, the ability of a plant to spread (the ease and range of the dispersal of seed) and persist (establish and reproduce in a new location) affects its likelihood of being classified as a weed.

In general, *Eucalyptus* species do not figure prominently as weeds around the world. This is perhaps surprising given that many eucalypts produce large quantities of seeds and possess diverse adaptations for dealing with disturbance. However, compared to species of *Pinus* (which have also enjoyed worldwide popularity as plantation trees), and members of the family Leguminosae, eucalypts are poor invaders (Richardson, 1998). Eucalypt seeds are generally small, but have no adaptation for dispersal and there is a high mortality amongst seedlings (see discussion earlier in this chapter). Due to their relatively long lifecycles, even under ideal circumstances they are slow to spread and establish, with the growth of many species being restricted by their preference for specific soils and climatic regimes. One study in Brazil, specifically designed to examine the abilities of *E. grandis* and *E. grandis* x *E. urophylla* to invade and establish in areas of

native vegetation lying adjacent to plantations, demonstrated that neither plant could effectively do so (da Silva et al., 2011). However, factors such as soil and climatic preferences do not altogether explain the comparatively poor invasive abilities of plants from this genus. The potential of mycorrhizal fungi for improving the establishment and performance of exotic eucalypts is still not fully explored (Chilvers et al., 2000; see above), but a lack of compatible ectomycorrhizal fungi has also been suggested as a factor limiting invasiveness (Rejmanek and Richardson, 2011).

Nevertheless, in a number of countries and environmental contexts, species of *Eucalyptus* have been classified as weeds. An Australian report prepared for the World Wildlife Fund lists five species (*E. botryoides*, *E. camaldulensis*, *E. citriodora*, *E. cladocalyx* and *E. maculata*) as posing a significant weed risk in some Australian states (Groves, Boden and Lonsdale, 2005). However, the online database from the Australian Weeds Committee National Initiative[8] records only *E. maculata* (spotted gum) as a weed anywhere in the country, while the database from the Australian Department of Environment[9] fails to list any *Eucalyptus* species. A study in Western Australia has reported that *E. megacornuta* has invaded areas of urban bushland, perhaps due to factors linked to the increased germination of seed and survival of seedlings after fire (Ruthrof, 2004).

Outside Australia, several species of *Eucalyptus* have been classified as weeds. The CABI Invasive Species Compendium[10] lists the following four *Eucalyptus* species as invasive: *E. sideroxylon*, *E. camaldulensis*, *E. cladocalyx* and *E. paniculata*. All four are reported to be invasive in South Africa and classified as category 2 under the South African Conservation of Agricultural Resources Act (1983). *E. camaldulensis* has been described as transforming large expanses of riverbanks in South Africa, and *E. grandis* and *E. lehmannii* have also been noted as weeds in that country (Forsyth et al., 2004). The Invasive Plant Atlas of the United States[11] designates six species as invasive of natural areas, including *E. camaldulensis* and *E. globulus*. Using the Australian Weed Risk Assessment System (Pheloung, Williams and Halloy, 1999), Gordon et al. (2012) evaluated the invasive potential of 38 species of *Eucalyptus* that, at the time of the study, were being tested or cultivated in the United States for pulp, biofuel or other purposes. It was found that 15 species (39%) had a low risk of invasion, 14 (37%) were high risk while the remainder needed further information to establish their status. The high risk species included *E. camaldulensis*, *E. globulus*, *E. grandis* and *E. saligna*, while the hybrid *E. urophylla* x *E. grandis* (*E. urograndis*) required further evaluation. In another study, Gordon et al. (2011) used the Australian Weed Risk Assessment System to explore the invasiveness of a range of plants that were under study as biofuels. Likewise, *Eucalyptus* species such as *E. camaldulensis* and *E. grandis* were concluded as having a high risk of invasiveness (Figure 6.16).

In both Nepal and South Africa, the invasion of areas by species of *Eucalyptus* has been linked to problems with the amount of water flowing in streams, and in turn the quantity of water in dams (Kunwar, 2003; Richardson and van Wilren, 2004). However, in some countries where *Eucalyptus* species have been extensively grown as a plantation crop for many years, such as Brazil and India, none have been classified as a weed (Pasquali, 2010; Reddy, 2008; Reddy et al., 2008).

When assessing the weediness of any given *Eucalyptus* species, it should not be forgotten that it relates to trees used for plantation in most cases. The invasive spread of trees from large-scale plantings (commercial plantations) into surrounding regions is usually greater than from areas under agroforestry practices (Richardson, 1998). This is

likely due to a number of factors, including the larger expanses of commercial plantations and the greater concern with the environment associated with agroforestry. Hence, the management practices of the plantations in question may form a major part of any weed risk assessment.

Figure 6.16. **Young *E. camaldulensis*, growing beside waterway at the Australian National Botanic Gardens**

Source: Courtesy Alison Wardrop, OGTR.

Control measures

Eucalyptus seedlings can be killed by the surface application of herbicides. In the case of adult trees, it is possible to drill holes around the perimeter of the trunk, or use an axe to place a series of cuts around the base; then a syringe is used to inject an herbicide, such as glyphosate, into the interior tissues. Climate and the time of the year are factors which must be kept in mind before using an herbicide. Burning, either by the controlled lighting of a fire around the target trees or the use of a flame gun, are also possible measures to kill trees. Large *Eucalyptus* trees can be felled by standard procedures, ideally this being coupled with the disposal of the timber, either as chips, mulch or its conversion to wooden products such as flooring. Ring barking (girdling), in which a strip of bark is removed all the way around a trunk, thus breaking the phloem tissue, can also be used to kill the upper portions of trees. This practice was commonly used to kill eucalypts on agricultural land during the early days of European settlement of Australia.

The clearing of a weed from an area is only the first step in its reclamation. Ideally, a linked strategy for the colonisation of the cleared area needs to be in place and acted upon, or else it is possible that another weed will take the place of the eradicated weed. For instance, when *E. grandis* was cleared from the banks of one river in South Africa, it was found that a group of unwanted weeds almost immediately sprouted to fill the ecological niche (Koenig, 2009).

Mating system and hybridisation in *Eucalyptus*

The possibility of genes transferring from any one of the *Eucalyptus* species to other organisms is addressed below. Potentially, genes could be transferred to: 1) plantation eucalypt populations; 2) other cultivated and naturalised eucalypt species; 3) other plant

genera; and 4) other organisms. For gene transfer beyond species, potential barriers must be overcome before gene flow can occur successfully. Pre-zygotic barriers include differences in floral phenology, different pollen vectors and different mating systems, such as stigmatic or stylar incompatibility systems. Post-zygotic barriers include genetic incompatibility at meiosis, selective abortion, lack of hybrid fitness, and sterile or unfit backcross progeny. Even where pre-zygotic and post-zygotic barriers do not exist, physical barriers created by geographic separation can still limit gene transfer to other plants.

Successful gene transfer requires that three criteria are satisfied. The plant populations must: 1) overlap spatially; 2) overlap temporally (including flowering duration within a year and flowering time within a day); and 3) be sufficiently close biologically that the resulting hybrids are fertile, facilitating introgression into a new population (den Nijs, Bartsch and Sweet, 2004).

Intraspecific crossing

As outlined above, *Eucalyptus* species are often capable of self-fertilisation, but in most cases breed by a combination of self-pollination and outcrossing, with a marked tendency to outcross (Pryor, 1976). This tendency is reinforced by protandry and by selection against the products of self-fertilisation in later stages of life; self-pollination often results in severe inbreeding depression for growth and survival, manifested as a reduction in capsule production, seed yield, and seedling growth and vigour compared with cross-pollination (Hardner and Potts, 1995; Potts, Hamilton and Blackburn, 2011; Potts and Wiltshire, 1997). Nonetheless, open pollinated seed collected from native stand and seed orchard trees still contain significant proportions of self-pollinated seed (Eldridge et al., 1994; Potts and Wiltshire, 1997).

Estimates of outcrossing rate

Quantitative estimates of outcrossing in several eucalypt species have been made on the basis of allozyme variants. From such studies, outcrossing has been shown to predominate, averaging about 75% in seed from natural populations of 18 species (Eldridge et al., 1994; Potts and Wiltshire, 1997). Subsequent estimates using values averaged over 23 species show a mean outcrossing rate of 0.74[12] (Byrne, 2008). Where comparisons have been made for species using both microsatellite markers and allozyme markers, it appears that the allozyme estimates may underestimate true outcrossing rates by up to 10% (Byrne, 2008). Estimates of the outcrossing rate in natural populations using rare morphological seedling markers range from 0.70 to 0.92 (McGowen et al., 2004), and in exotic stands the range is 0.62-0.90.

For individual species, estimates of outcrossing in native populations of *E. globulus* range from 0.65 to 0.89 (Mimura et al., 2009) and in seed orchards from 0.60 to 0.90 (Potts et al., 2008). McGowen et al. (2004) used a single locus morphological marker to estimate outcrossing in *E. globulus* and suggested that pollinator activity and flower abundance had little effect on outcrossing rate, rather the self-incompatibility of a tree is probably the primary determinant of its outcrossing rate.

Only seed orchard estimates of outcrossing rates have been published for *E. nitens*, and these range from 0.75 to 0.87 (reviewed in Grosser, Potts and Vaillancourt, 2010). This is similar to outcrossing rates estimated in natural seed orchards and breeding populations of other eucalypt species: *E. camaldulensis*: 0.75; *E. regnans*: 0.91;

E. urophylla: 0.89-0.93 (Jones et al., 2008), and *E. grandis*: 0.84 (House, 1997; James and Kennington, 1993).

Self-incompatibility

Self-incompatibility has been studied in only a few species of *Eucalyptus*. These studies indicate that there may be more than one self-incompatibility mechanism in eucalypts and that both pre- and post-zygotic mechanisms may operate (Ellis and Sedgley, 1992; Horsley and Johnson, 2007; McGowen et al., 2010; Pound et al., 2003a, 2003b, 2002a, 2002b; Sedgley and Granger, 1996; Sedgley et al., 1989; Sedgley and Smith, 1989).

In *E. globulus*, self-incompatibility is probably the primary determinant of outcrossing rate rather than pollinator activity or flower abundance (McGowen et al., 2010, 2004; Patterson et al., 2004). Self-incompatibility in this species is estimated at 87-89% and is thought to be mainly due to late-acting mechanisms operating in the ovary, with post-zygotic abortion of self-fertilised ovules (Pound et al., 2002a). Similarly, ovule breakdown has been suggested in *E. nitens* as a late-acting self-incompatibility response (Pound et al., 2003b). Studies of the breeding systems of *E. urophylla* and *E. grandis* suggest that, in addition to a late-acting self-incompatibility barrier, cryptic self-incompatibility in the form of self-pollen tube growth retardation could be responsible for the preferential outcrossing observed for these two species (Horsley and Johnson, 2007).

Natural and manipulated hybridisation

A comprehensive overview of natural and manipulated hybridisation patterns within the genus *Eucalyptus* L'Hérit can be found in reviews by Griffin, Burgess and Wolf (1988) and Pryor and Johnson (1981; 1971). In addition, Potts, Barbour and Hingston (2001) and Potts et al. (2003) have compiled a large volume of published work on the characteristics of plantation eucalypt species and hybrids, in the context of assessing the risk of genetic pollution from farm forestry. A comprehensive list of reports relating to natural and manipulated hybrids of the major plantation *Eucalyptus* species was tabulated in Potts, Barbour and Hingston (2001) and includes a vigour rating for hybrid seedlings; extracts from that publication are reproduced in Annex 6.A1. In addition, the potential for gene flow from exotic eucalypt plantations into Australian native eucalypts has been explored by Barbour et al. (2010). Some of the key conclusions and summaries from those reports are included in the discussion below.

The degree to which hybridisation may occur is limited by pre-mating barriers such as spatial isolation and flowering asynchrony (Keatley, Hudson and Fletcher, 2004; Potts and Wiltshire, 1997), and by post-mating crossing incompatibilities. Pollination mechanisms are a major determinant of gene flow in plants; species which are located, by distance or other physical features, beyond the normal range of pollen transfer are unlikely to hybridise (Duncan, 1989). This is particularly the case for eucalypts, for which gene flow by seed dispersal is quite limited (see above; Byrne, 2008). Pollen transfer between eucalypts occurs via the activities of non-specific biotic vectors such as birds and insects rather than wind, and the extent of pollen dispersal is influenced by the type and efficiency of pollinators (see above).

Other determinants of gene flow are: 1) season of flowering (phenology); and 2) lack of reproductive compatibility. Seasonal differences in flowering time are one of the major pre-zygotic barriers to gene flow within *Eucalyptus* (Drake, 1980; Pryor, 1976). For

inter-provenance crossing in a seed orchard of *E. regnans*, for example, differences in peak flowering time of only two weeks was enough to reduce crossing to 65% of that expected under random mating (in Potts, Barbour and Hingston, 2001). However, flowering within eucalypt species may be highly variable and influenced by numerous other factors (Eldridge et al., 1994; House, 1997; Potts and Wiltshire, 1997). Most eucalypts display protandrous flower development and, because pollen is usually shed before the eucalypt stigma becomes receptive, late-flowering trees are more likely to pollinate early-flowering trees (see above). A summary of relative flowering times in Australia for a range of plantation species can be found in Table 5.3 of Potts, Barbour and Hingston (2001).

In addition to premating barriers such as geographic isolation and flowering asynchrony, post-mating crossing incompatibilities will also determine the level of gene flow. Controlled crossing experiments have shown that there are two major pre-zygotic barriers to hybridisation. The first is a structural barrier which is unilateral, and due to the pollen tubes of small-flowered species being unable to grow the full length of the style of large-flowered species (see above; Gore et al., 1990). The resulting reduction in seed set has hindered attempts to produce F_1 hybrids between *E. globulus* and smaller flowered species such as *E. gunnii*, *E. camaldulensis*, *E. nitens*, *E. grandis* or *E. dunnii*. However, since flower and style size are inherited in an intermediate manner, once F_1 hybrids are obtained, the physical barrier between species can be broken down (Potts, Barbour and Hingston, 2001).

The second barrier is physiological and results in pollen tube abnormalities and pollen tube arrest in the pistil. This prevents successful hybridisation between the three genera of eucalypts (*Angophora*, *Corymbia* and *Eucalyptus*), as well as between the major subgenera within *Eucalyptus* (Griffin, Burgess and Wolf, 1988; Potts et al., 2003; Pryor and Johnson, 1971).

Natural hybridisation

Griffin, Burgess and Wolf (1988) examined patterns of both natural and manipulated hybridisation within the genus *Eucalyptus* and, consistent with earlier work by Pryor and Johnson (1971; 1981), found that the occurrence of hybrid combinations reflects the degree of taxonomic distance. Barriers to hybridisation between species within subgenera are often weak (Griffin, Burgess and Wolf, 1988; Hardner and Potts, 1995; Potts et al., 2003), and natural hybridisation and introgression between recognised taxa is relatively common (Butcher and Williams, 2002; Field et al., 2011; Griffin, Burgess and Wolf, 1988; Potts and Gore, 1995; Potts and Wiltshire, 1997). In decreasing order of frequency, hybrids are found to occur within series, between series and between sections. Thus, interspecific hybridisation between species from the same section is commonly reported, but hybridisation between species from the major subgenera or genera does not occur.

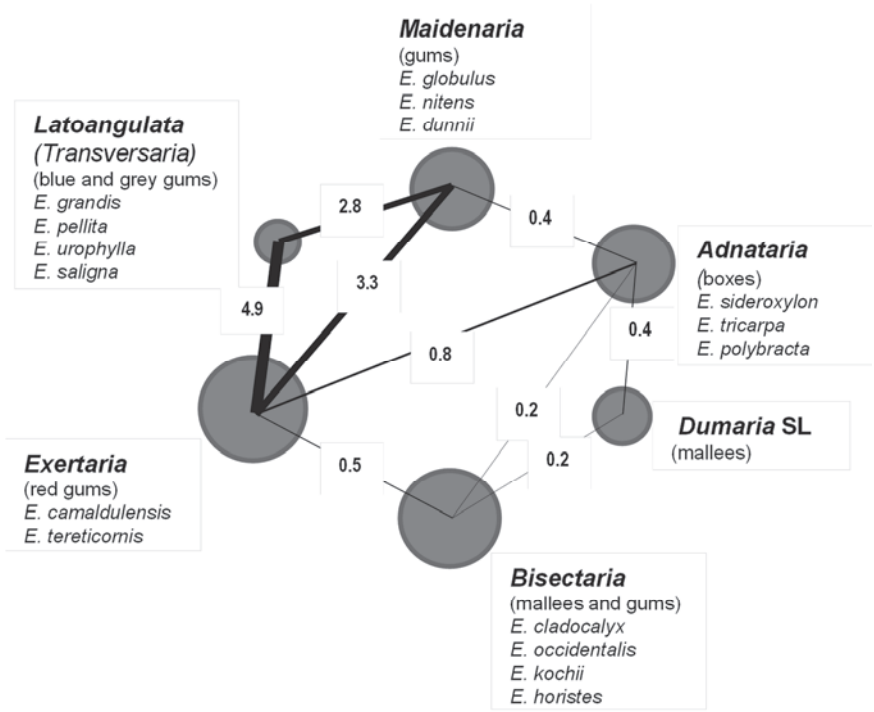
Natural hybridisation may be rather restricted (Griffin, Burgess and Wolf, 1988) since, amongst recorded natural hybrids only 15% of combinations expected on geographic and taxonomic grounds had been recorded. Nonetheless, it appears that in native forest there is a low background level of natural crossing continually occurring between species. In Australian native forests, this background level of F_1 hybridisation was found to average 1.62% across 13 species, and from 0.03% to 3.5% at the individual species level (see Table 3 in Potts et al., 2003). While it has been suggested that human activity may have enhanced this rate of hybrid formation and survival (e.g. through the introduction of honey bees and habitat disturbance), there is no doubt that hybridisation in

the genus is natural and has been a significant factor in eucalypt evolution (Potts, Barbour and Hingston, 2001).

Within and between sections

The occurrence of natural and artificial hybrids of the main *Symphyomyrtus* plantation eucalypt species (data from Griffin, Burgess and Wolf, 1988) is summarised in Annex 6.A1, and the frequency of inter-sectional hybridisation is shown diagrammatically in Figure 6.17. Only 40 natural intersectional hybrids were reported in *Symphyomyrtus*.

Figure 6.17. **Natural inter-sectional hybridisation in *Symphyomyrtus***



Note: The figure shows the frequency of natural inter-sectional hybrids as a percentage of the number of intersectional combinations possible amongst proximal species (within 10 x 10 minutes of longitude and latitude). The area of the circle indicates the number of species in each section. Sections follow Pryor and Johnson (1971). Section *Transversaria* was renamed *Latoangulatae* by Brooker (2000).

Source: Adapted from Potts et al. (2003) (reproduced from Steane et al. (2002)).

The summarised data suggest that species from *Exertaria* (e.g. *E. camaldulensis*) can potentially hybridise with all other major sections except *Dumaria*. Plantation species from the *Latoangulatae* (e.g. *E. grandis*, *E. pellita*) are more likely to hybridise with species from the *Exertaria* or *Maidenaria* than with other sections of *Symphyomyrtus*. No natural hybrids have been reported between *Maidenaria* and either *Bisectaria* or *Dumaria* species.

Within the section *Maidenaria*, the potential for natural hybridisation of eucalypts from plantations and native forests has been documented by Barbour, Potts and Vaillancourt (2003; 2005) and Barbour et al. (2002) on the island of Tasmania. Hybrids between *E. ovata*, which is native to the island, and the introduced plantation species

E. nitens, were found in a number of locations, and it was concluded that such hybrids were establishing in the wild (Barbour, Potts and Vaillancourt, 2003; Barbour et al., 2002). Further studies of pollination from *E. nitens* plantations showed that *E. ovata* plants within 100 m of *E. nitens* produced approximately 7% hybrids, but after 200 m the number of hybrids had dwindled to less than 1% per plant (Barbour, Potts and Vaillancourt, 2005).

Between genera and subgenera

Post-mating crossing incompatibility prevents successful hybridisation between the three genera of eucalypts (*Angophora*, *Corymbia* and *Eucalyptus*), as well as between the major subgenera within *Eucalyptus* (Griffin, Burgess and Wolf, 1988; Potts et al., 2003). Only two records were found of imputed natural hybrids between *Eucalyptus* subgenera; both proved to be misidentifications when re-examined (Griffin, Burgess and Wolf, 1988).

Manipulated hybridisation

Controlled pollination, or manipulated/artificial hybridisation, is used for the generation of interspecific hybrids for plant improvement (Eldridge et al., 1994). It has been widely used as a breeding strategy in eucalypts in subtropical and tropical regions of the world, but to a lesser extent in temperate regions (Harwood, 2011; Potts and Dungey, 2004).

Most records of manipulated hybrids are derived from the subgenus *Symphyomyrtus*. As part of a review of the risks of genetic pollution from planting non-native eucalypt species and hybrids in Australia, Potts, Barbour and Hingston (2001) and Potts et al. (2003) summarised Griffin's data and supplemented it with new records of manipulated and artificial hybridisation. Annex 6.A1 is adapted from tabulated data presented in Potts, Barbour and Hingston (2001), and some of the major conclusions drawn in that review are outlined below.

Within sections

Reports of successful manipulated interspecific hybridisation within sections are relatively common (Delaporte, Conran and Sedgley, 2001; Griffin, Burgess and Wolf, 1988) (see Annex 6.A1), some of the hybrids having become of significant commercial importance. *E. grandis* x *E. urophylla* hybrids are planted extensively in Brazil (Goncalves et al., 2008; Potts and Dungey, 2004). In Australia and South America, there has been considerable research on artificial hybridisation of the major plantation species *E. nitens* and *E. globulus*, which would not normally hybridise due to temporal and geographic barriers (Potts and Dungey, 2004; Potts et al., 2000; Tibbits, 2000). Hybrid clones between these species have been developed in Chile (Griffin et al., 2000), but attempts in Australia have been unsuccessful, in part due to the inability to achieve clonal propagation (Potts, Hamilton and Blackburn, 2011).

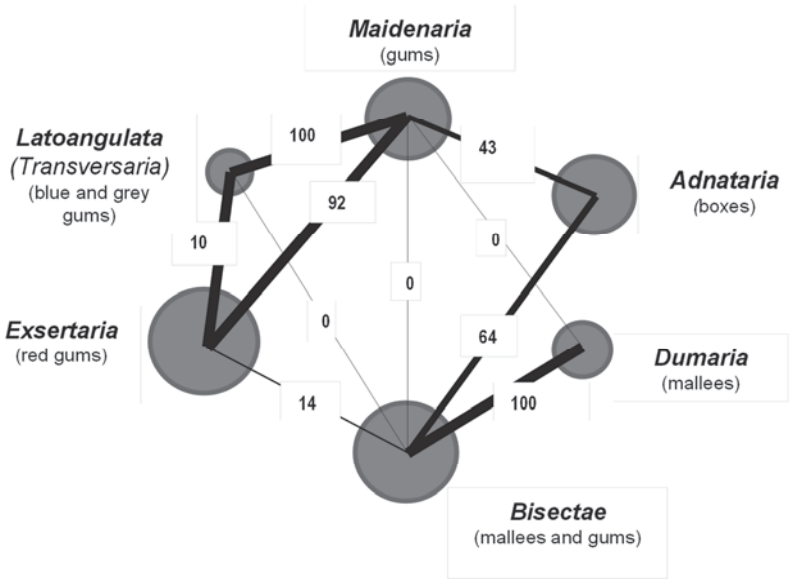
Between sections

Data from manipulated hybridisation studies are consistent with the observations outlined for natural hybrids (see previous section and Figure 6.18.). Thus, within *Symphyomyrtus*, it appears that plantation species from the section *Exertaria* can potentially hybridise with all other major sections except possibly the mallees (Griffin, Burgess and Wolf, 1988; Potts, Barbour and Hingston, 2001). Plantation species from the

section *Latoangulata* (*Transversaria*) are more likely to hybridise with species from either the *Exertaria* or *Maidenaria* than the other *Symphyomyrtus* sections. Overall, *Latoangulata* has the highest number of intersectional hybrids.

Delaporte, Conran and Sedgley (2001) reported on 36 individual crosses between series in section *Bisectaria* and between section *Bisectaria* and section *Adnataria*. The results confirmed and extended earlier findings that crosses between species from *Bisectaria* and *Adnataria* have a relatively high chance of success (Ellis, Sedgley and Gardner, 1991). This study also confirmed that crosses between closely related species have a greater degree of success than those between distant crosses, as do those between species with similar flower size (Delaporte, Conran and Sedgley, 2001). The resulting hybrid seedlings displayed leaf and stem characteristics that were intermediate between the maternal and pollen parents, albeit closer to the maternal parent.

Figure 6.18. **Manipulated inter-sectional hybridisation in *Symphyomyrtus***



Note: The figure shows the percentage of successful species combinations produced from manipulated inter-sectional cross-combinations within *Symphyomyrtus*. Sections follow Pryor and Johnson (1971). Also see Annex 6.A1.

Source: Adapted from Potts et al. (2003).

Even if interspecific mating occurs and seed is produced, it is not certain that hybrids will survive and introgression will occur. Hybrid offspring are also usually intermediate between parental taxa for flowering time and physical characteristics such as flower size (Lopez et al., 2000; McComb et al., 2000) and this may potentially enhance backcrossing to parental species. However, progeny derived from hybrids crossing to either of the parental species or an unrelated third species are likely to exhibit loss of fitness due to advanced generation breakdown (outbreeding depression). Some interspecific crosses of *Symphyomyrtus* are reported to be more susceptible to insects and fungal pests than parental species (Harwood, 2011) and impaired reproductive capability, abnormalities and dwarfism are common features of many later generation eucalypt species (Delaporte, Conran and Sedgley, 2001; Pilipenko, 1969; Potts, Barbour and Hingston, 2001).

Between genera and subgenera

There are a few reports of manipulated intersubgeneric hybrids, but seedlings from such crosses either died or showed very poor vigour or results need further validation (Ladiges, 1997; Meddings et al., 2003). In documenting extensive work in the Russian Federation on eucalypt hybridisation, Pilipenko (1969) mentioned 17 combinations of manipulated hybrids between subgenera. Of these, 13 were unsuccessful in that they produced no viable seed.

Human health

Potential allergens and toxins from *Eucalyptus* are the products of flowers, especially pollen, gums from stems and branches, and the oils that are extracted from leaves.

Eucalyptus pollen can cause an allergic reaction in many people, but it is not considered a severe problem. One Internet database, providing details of the allergenicity of pollen from various plants native and introduced to the United States, lists four *Eucalyptus* species, and in each case classifies their pollen as a mild allergen.[13] In India, although pollen from *Eucalyptus* has been commonly found, it is not regarded as of allergenic importance (Singh and Dahiya, 2008). Allergenic symptoms from pollen include irritation to the respiratory tract and skin, conjunctivitis, asthma, nasal congestion and even malfunction of the vocal cords. Such symptoms in susceptible individuals are invariably seasonal, corresponding to the time when the relevant *Eucalyptus* trees release pollen, but environmental factors such as the level of humidity can influence the responses of people.

The oils from *Eucalyptus* leaves have long been extracted and used in various commercial and medicinal capacities. In medicine, such oils have been used to relieve the symptoms of respiratory tract infections and inflammations, and reduce the effects of asthma (Juergens et al., 2003).

Although eucalyptol can be ingested in small quantities (such as in mouthwash and through application to the nose and other parts of the skin), it is toxic when consumed in high dose, with an oral LD50 in rat of 2 480 mg/kg. It is oxidised by cytochrome p450 enzymes to one of two metabolites which are then excreted in urine (Duisken et al., 2005). Rarely, application to the skin of *Eucalyptus* oil (which often consists mostly of eucalyptol) can induce significant symptoms of toxicity. In one case, the use of *Eucalyptus* oil to treat urticaria (hives) induced severe nervous system toxicity, evidenced by slurred speech, muscle weakness and unconsciousness (Darben, Cominos and Lee, 1998). In addition, eucalyptol has been recorded as producing an allergic response in some people, usually characterised by a rash and the desire to scratch the infected areas (Vilaplana and Romaguera, 2000). Use of a corticosteroid can relieve the symptoms.

Notes

1. This citation was added as an update in January 2016.
2. This citation was added as an update in January 2016.
3. This citation was added as an update in January 2016.
4. This citation was added as an update in January 2016.
5. Clinal variation: continuous variation in form between individual leaves.
6. Protandry: anthers dehisce and shed pollen before the stigma becomes receptive.
7. *www.phytozome.net/eucalyptus.php*.
8. Weeds Australia website at: *www.weeds.org.au*.
9. Weeds Australia website at: *www.weeds.org.au.*
10. CABI Invasive Species Compendium website at: *www.cabi.org/isc*.
11. Invasive Plant Atlas of the US website at: *www.invasiveplantatlas.org/index.html*.
12. t; 0 = complete self-fertilisation, 1 = complete outcrossing.
13. *www.pollenlibrary.com*.

Annex 6.A1. Compilation of natural and manipulated hybrids of major eucalypt plantation species[1]

Vigour rating (V)

- 1: apparently healthy seedlings or trees
- 2: below mid parent performance noted
- 3: some vigorous but also others with viability problems
- 4: successful seed set and early seedling growth but failed to survive in later years
- 5: seedlings or trees with stunted growth
- 6: fruit set or seed only
- 7: failed hybrid combinations
- S: successful seed set only reported, not planted

Female/male (f/m): whether the species listed was used as the female or male in the cross.

Table 6.A1.1. ***Eucalyptus globulus***

A. Natural hybrids

Species	Reference
E. barberi	Williams and Potts (1996)
E. brookeriana	Williams and Potts (1996)
E. ovata	Jordan et al. (1993); Williams and Potts (1996)
E. kitsoniana	Griffin, Burgess and Wolf (1988)
E. goniocalyx	Griffin, Burgess and Wolf (1988)
E. nortonii	Griffin, Burgess and Wolf (1988)
E. cypellocarpa	Kirkpatrick, Simmons and Parsons (1973)
E. pseudoglobulus	Griffin, Burgess and Wolf (1988)
E. bicostata	Griffin, Burgess and Wolf (1988)
E. johnstonii	Griffin, Burgess and Wolf (1988)
E. viminalis	Griffin, Burgess and Wolf (1988)
E. cordata	Williams and Potts (1996)
E. rubida	Griffin, Burgess and Wolf (1988)
E. urnigera	Griffin, Burgess and Wolf (1988)
E. perriniana	Williams and Potts (1996)

1. Adapted from Potts, Barbour and Hingston (2001) and based on data from Griffin, Burgess and Wolf (1988).

Table 6.A1.1. ***Eucalyptus globulus*** (*continued*)

B. Manipulated hybrids

Species	V	FM	Reference
E. urophylla	3	f	Griffin et al. (2000)
E. grandis	3	f	Griffin et al. (2000)
E. robusta	1		Griffin, Burgess and Wolf (1988)
E. pellita			D. Boomsma personal communication (in Potts, Barbour and Hingston, 2001)
E. longifolia			Griffin, Burgess and Wolf (1988)
E. loxophloeba			Griffin, Burgess and Wolf (1988)
E. camaldulensis	3	f	McComb et al. (2000); Mesbah (1995); Sasse, George and Dale (2000)
E. dunnii	3	f	Griffin, Burgess and Wolf (1988) ; Griffin et al. (2000); Barbour and Spencer (2000) (cut style)
E. nitens	3	f	Griffin et al. (2000); Potts et al. (2000)
E. maidenii	1	m	Potts unpublished data
E. bicostata	1	f	Potts unpublished data
E. viminalis	1		Griffin, Burgess and Wolf (1988)
E. gunnii	1	f	Potts et al. (2000)
E. sideroxylon	7	f	Griffin, Burgess and Wolf (1988)

Table 6.A1.2. ***Eucalyptus nitens***

A. Natural hybrids

Species	Reference
E. quandrangulata	Tibbits, Boomsma and Jarvis (1997)

B. Manipulated hybrids

Species	V	FM	Reference
E. grandis	3	f	Shelbourne, Hong and McConnochie (1999) Verryn (2000)
	2		Tibbits (2000)
E. saligna	2	m	Tibbits (2000)
E. botryoides	4	m	Tibbits (2000)
E. oldfieldi	7	m	Tibbits (2000)
E. camaldulensis	3	m	Tibbits (2000; 1989; 1988)
	3	m	
E. rudis	4	m	Tibbits (2000)
E. ovata	1	m	Tibbits (2000)
E. rodwayi	2	m	Tibbits (2000)
E. neglecta	2	m	Tibbits (2000)
E. parvifolia	1	m	Tibbits (2000)
E. dunnii	2	m	Tibbits (2000)
E. cypellocarpa			Griffin, Burgess and Wolf (1988)
E. globulus	3	m	Griffin et al. (2000);
		m	Potts et al. (2000);
		m	Tibbits (2000; 1989; 1988)
E. quandrangulata	6	m	Tibbits (2000)
E. johnstonii	1	m	Tibbits (2000)
E. macarthurii	6	m	Tibbits (2000)
E. viminalis	1	m	Tibbits (2000; 1989; 1988)
E. dalrympleana	1	m	Tibbits (2000)
E. rubida	1	m	Tibbits (2000)
E. glaucescens	7	m	Tibbits (2000)

B. Manipulated hybrids (*continued*)

Species	V	FM	Reference
E. gunnii	1	mf	Tibbits (2000; 1989; 1988)
E. morrisbyi	2	m	Tibbits (2000)
E. urnigera	S	m	Tibbits (2000)
E. perriniana	2	mf	Tibbits (2000)
E. cordata	1	m	Tibbits (2000)
E. decipiens	7	m	Tibbits (2000)
E. gillii	7	m	Tibbits (2000)
E. incrassata	7	m	Tibbits (2000)
E. tereticornis	7	m	Tibbits (2000)
E. pulverulenta	7	m	Tibbits (2000)
E. lansdowneana	7	m	Tibbits (2000)
E. fibrosa	7	m	Tibbits (2000)

Table 6.A1.3. ***Eucalyptus grandis***

A. Natural hybrids

Species	Reference
E. saligna	Griffin, Burgess and Wolf (1988)
E. robusta	Griffin, Burgess and Wolf (1988)
E. pellita	Griffin, Burgess and Wolf (1988)
E. tereticornis	Griffin, Burgess and Wolf (1988)

B. Manipulated hybrids

Species	V	FM	Reference
E. urophylla	1		de Assis (2000); Vigneron and Bouvet (2000); Wu, Wu and Xu (1996)
	1		D. Boomsma personal communication (in Potts, Barbour and Hingston, 2001)
E. botryoides			D. Boomsma personal communication (in Potts, Barbour and Hingston, 2001)
E. pellita	1		Griffin, Burgess and Wolf (1988)
	1		de Assis (2000)
E. alba	1	f	Griffin, Burgess and Wolf (1988)
E. tereticornis	1		Griffin, Burgess and Wolf (1988); Verryn (2000); Vigneron and Bouvet (2000)
E. camaldulensis	1		Griffin, Burgess and Wolf (1988)
	1		de Assis (2000)
			Verryn (2000)
	1	f	Dale, Aitken and Sasse (2000); Sasse, George and Dale (2000)
E. dunnii	3	fm	Griffin et al. (2000)
	3		de Assis (2000)
E. nitens	3	m	Shelbourne, Hong and McConnochie (1999); Tibbits (2000); Verryn (2000)
	2		
E. maidenii		fm	D. Boomsma personal communication (in Potts, Barbour and Hingston, 2001)
	3		de Assis (2000)
E. globulus	3	m	Griffin et al. (2000)
E. gunnii	3	f	Potts unpublished data (in Potts, Barbour and Hingston, 2001)
E. pulverulenta			Paton (1981)
E. leucoxylon	7	m	Griffin, Burgess and Wolf (1988)
E. resinifera			David Lee personal communication (in Potts, Barbour and Hingston, 2001)

Table 6.A1.4. ***Eucalyptus pellita***

A. Natural hybrids

Species	Comments	Reference
E. grandis		Griffin, Burgess and Wolf (1988)
E. resinifera		Griffin, Burgess and Wolf (1988)
E. punctata		Griffin, Burgess and Wolf (1988)
E. brassiana		Harwood (1998)

B. Manipulated hybrids

Species	V	FM	Reference
E. deglupta	7	m	Griffin, Burgess and Wolf (1988)
	5		Vigneron and Bouvet (2000)
	1?		Sariot (2013)
E. urophylla	1	fm	Harwood (1998); Vigneron and Bouvet (2000)
	3		de Assis (2000); Wu, Wu and Xu (1996)
E. deanei			D. Boomsma personal communication (in Potts, Barbour and Hingston, 2001)
E. grandis	1		Griffin, Burgess and Wolf (1988)
	1		de Assis (2000)
E. alba	6	f	Griffin, Burgess and Wolf (1988)
E. tereticornis	1	f	Griffin, Burgess and Wolf (1988)
E. camaldulensis			Harwood (1998)
E. maidenii			D. Boomsma personal communication (in Potts, Barbour and Hingston, 2001)
E. globulus			D. Boomsma personal communication (in Potts, Barbour and Hingston, 2001)

Table 6.A1.5. ***Eucalyptus dunnii***

A. Natural hybrids

Species	Comments	Reference

B. Manipulated hybrids

Species	V	FM	Reference
E. urophylla		f	Griffin et al. (2000)
			D. Boomsma personal communication (in Potts, Barbour and Hingston, 2001)
E. grandis	3	mf	Griffin et al. (2000)
	3		de Assis (2000)
E. maidenii	1		de Assis (2000)
E. globulus	3	m	Griffin et al. (2000)
		f	Barbour and Spencer (2000) (cut style)

Table 6.A1.6. ***Eucalyptus camaldulensis***

A. Natural hybrids

Species	Comments	Reference
E. robusta	Spontaneous in exotic plantations	Kha and Cuong (2000)
E. cladocalyx		Griffin, Burgess and Wolf (1988)
E. alba		Griffin, Burgess and Wolf (1988)
E. bigalerita		Griffin, Burgess and Wolf (1988)
E. tereticornis		Griffin, Burgess and Wolf (1988)
E. blakelyi		Griffin, Burgess and Wolf (1988)
E. dwyeri		Griffin, Burgess and Wolf (1988)
E. rudis		Griffin, Burgess and Wolf (1988)
E. ovata		Griffin, Burgess and Wolf (1988)
E. bridgesiana		Griffin, Burgess and Wolf (1988)
E. viminalis		Griffin, Burgess and Wolf (1988)
E. largiflorens		Griffin, Burgess and Wolf (1988)
E. melliodora		Griffin, Burgess and Wolf (1988)
E. leucoxylon		Griffin, Burgess and Wolf (1988)

B. Manipulated hybrids

Species	V	FM	Reference
E. diversicolor		m	Mesbah (1995)
E. grandis	1		de Assis (2000) Mesbah (1995)
	1	m	Dale, Aitken and Sasse (2000); Sasse, George and Dale (2000)
E. botryoides	1		Griffin, Burgess and Wolf (1988)
E. cladocalyx		m	Mesbah (1995)
E. urophylla	1		de Assis (2000)
	1		Kha and Cuong (2000); Wu, Wu and Xu (1996)
E. tereticornis	1	m	Griffin, Burgess and Wolf (1988) Mesbah (1995)
E. blakelyi	1	f	Griffin, Burgess and Wolf (1988)
E. macarthurii	1	f	Griffin, Burgess and Wolf (1988)
E. viminalis	1	fm	Griffin, Burgess and Wolf (1988)
E. exerta	1		Kha and Cuong (2000)
E. maidenii		m	Mesbah (1995)
E. globulus	3	m	McComb et al. (2000) Sasse, George and Dale (2000)
E. gunnii	5	mf	Griffin, Burgess and Wolf (1988)
E. laevopinea	7	f	Griffin, Burgess and Wolf (1988)
E. fastigata	7	f	Griffin, Burgess and Wolf (1988)

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List of OECD consensus documents on environmental safety assessment, 1996-2015

	Consensus document	Lead country(ies)	Year of issue	Volume
Facilitating harmonisation	Designation of a Unique Identifier for Transgenic Plants (revised version) (guidance document)	Working Group	2006	Vol. 3
	Introduction to the OECD Biosafety Consensus Documents (*available in English and French)*	Working Group	2005	Vol. 1, 3, 4, 5 & 6
	Low-Level Presence of Transgenic Plants in Seed and Grain Commodities: Environmental Risk/Safety Assessment, and Availability and Use of Information	Working Group	2013	Vol. 6
	Molecular Characterisation of Plants Derived from Modern Biotechnology	Canada	2010	Vol. 3
	Points to Consider for Consensus Documents on Biology of Cultivated Plants	Working Group	2006	Vol. 3
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 and Netherlands	1999	Vol. 1
	Genes and their Enzymes that Confer Tolerance to Phosphinothricin Herbicide	United States, Germany and 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	**Information Used in the Assessment of Environmental Applications of Micro-organisms**			
	Acidithiobacillus	Canada	2006	Vol. 2
	Acinetobacter	Canada	2008	Vol. 4
	Baculovirus	Germany	2002	Vol. 2
	Pseudomonas	United Kingdom	1997	Vol. 2
	Guidance Documents on Biosafety Aspects of Bacteria			
	Horizontal Gene Transfer Between Bacteria	Germany	2010	Vol. 4
	Methods for Detection of Micro-organisms Introduced into the Environment: Bacteria	Netherlands	2004	Vol. 4
	Use of Information on Pathogenicity Factors: Bacteria	Netherlands and Canada	2011	Vol. 5
	Use of Taxonomy in Risk Assessment of Micro-organisms: Bacteria	Canada and United States	2003	Vol. 4
Biology of crops	Bananas and plantains (*Musa* spp.)	Spain	2009	Vol. 4
	Brassica crops (*Brassica* spp.)	Canada	2012	Vol. 5
	Cassava (*Manihot esculenta*)	Brazil, NEPAD-ABNE and ILSI-CERA	2014	Vol. 6
	Chili, hot and sweet peppers (*Capsicum annuum*)	Korea, Mexico and United States	2006	Vol. 1

	Consensus document	Lead country(ies)	Year of issue	Volume
Biology of crops (*continued*)	Common bean (*Phaseolus vulgaris*)	Brazil and ILSI-CERA	2015	Vol. 6
	Cotton (*Gossypium* spp.)	Spain	2008	Vol. 4
	Cowpea (*Vigna unguiculata*)	Australia	2015	Vol. 6
	Maize (*Zea mays* subs. *mays*)	Mexico	2003	Vol. 1
	Squashes, pumpkins, zucchinis and gourds (*Cucurbita*)	Mexico and United States	2012	Vol. 5
	Oyster mushroom (*Pleurotus* spp.)	Korea	2005	Vol. 1
	Papaya (*Carica papaya*)	United States	2005	Vol. 1
	Potato (*Solanum tuberosum* subsp. *tuberosum*)	Netherlands and United Kingdom	1997	Vol. 1
	Rice (*Oryza sativa*)	Japan	1999	Vol. 1
	Oilseed rape (*Brassica napus*): ***replaced with* Brassica *Crops (2012) in Vol. 5***	Canada	1997	Vol. 1
	Sugar beet (*Beta vulgaris*)	Switzerland	2001	Vol. 1
	Sugarcane (*Saccharum* spp.)	Australia	2013	Vol. 6
	Sunflower (*Helianthus annus*)	France	2004	Vol. 1
	Wheat (*Triticum aestivum*)	Germany	1999	Vol. 1
	Soybean (*Glycine max*)	Canada	2000	Vol. 1
Biology of trees	***Timber trees***			
	Birch: European white birch (*Betula pendula*)	Finland	2003	Vol. 2
	Douglas fir (*Pseudotsuga menziesii*)	Canada	2008	Vol. 3
	Eucalyptus (*Eucalyptus* spp.)	Australia	2014	Vol. 6
	Larches : North American larches (*Larix lyalli, Larix occidentalis, Larix laricina*)	Canada	2007	Vol. 3
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	Pines: Jack pine (*Pinus banksiana*)	Canada	2006	Vol. 3
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	Spruces: Norway spruce (*Picea abies*)	Norway	1999	Vol. 2
	Spruces: Sitka spruce (*Picea sitchensis*)	Canada	2002	Vol. 2
	Spruces: White spruce (*Picea glauca*)	Canada	1999	Vol. 2
	Fruit trees			
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	Papaya (*Carica papaya*) [listed above in “Crops”]	United States	2005	Vol. 1
	Stone fruits (*Prunus* spp.)	Austria	2002	Vol. 2

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Harmonisation of Regulatory Oversight in Biotechnology

Safety Assessment of Transgenic Organisms in the Environment, Volume 6

OECD CONSENSUS DOCUMENTS

Volume 6 of this Series compiles the science-based consensus documents issued by the OECD Working Group on the Harmonisation of Regulatory Oversight in Biotechnology from 2013 to 2015. They contain information for use during the risk/safety assessment of transgenic organisms to be released in the environment, for agriculture, forestry or other purposes. The first chapter deals with the low-level presence of transgenic plants in seed and grain commodities and how this knowledge can be used in biosafety regulatory assessment. The following chapters cover the biology of several crop species (sugarcane, cassava, common bean and cowpea) and include elements of taxonomy, centres of origin, reproductive biology, genetics, hybridisation and introgression, crop production and cultivation practices, interactions with other organisms such as pests and pathogens, and biotechnological developments. The last chapter relates to the biology of eucalyptus tree, focused on those species and hybrids which are planted commercially and might be the subject of genetic modification. This volume should be of value to applicants for commercial uses of transgenic organisms, regulators and risk assessors in national authorities, as well as the wider scientific community.

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