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**OECD SCHEMES FOR THE VARIETAL CERTIFICATION OF SEED MOVING IN INTERNATIONAL TRADE**

# GUIDELINES

# FOR CONTROL PLOT TESTS AND FIELD INSPECTION OF SEED CROPS

**ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT**

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## INTRODUCTION

1. The OECD Schemes for the varietal certification of seed moving in international trade, hereafter called the "OECD Seed Schemes", are a set of procedures, methods and techniques which enables monitoring of the quality of seed during the multiplication process and which ensure that both the varietal identity and the varietal purity of varieties is maintained and safeguarded.

2. Various checks are made at different stages of seed production to ensure that mechanical admixtures, mutations, out-pollination by undesirable pollen and other unforeseen occurrences have not prejudiced the quality of the seed in any way.

3. In order to do this, the characteristics which distinguish one variety from another have to be established so that it is possible to identify seed crops and seed lots as being consistent with the known characteristics of the variety recognised at the time of Registration. These characteristics are used not only for confirming varietal identity or trueness to type but also varietal purity; they need to be suitable for use in field conditions, although there are also a few characters which in some species relate to the seed itself.

4. The assessment of varietal identity and purity during seed production is crucial for maintaining high standards of seed quality.

5. The seed producer must ensure that nothing happens during the growing of the seed crop, the harvesting operation, the processing, bagging and labelling of the seed lot or the subsequent distribution which could adversely affect seed quality.

6. The OECD Seed Schemes provide two procedures which are designed to check the progress of a variety at different stages in the seed production process. These are as follows:

(a) Tests on plants grown in control plots, and laboratory tests on seeds and seedlings, using samples of seed drawn from lots ;

(b) Field inspection of growing seed crops, on one or more occasions, to report on their condition.

In making these tests and inspections it is essential to adopt technical methods which will achieve results of sufficient accuracy and reliability, yet can be operated within the limits of available resources.

7. The methods described in these Guidelines have been found, over a period of many years, to give satisfactory results and provide the principles upon which such methods should be based. They have been adopted by most OECD Member and Non member countries participating in the OECD Seed Schemes and exchanging certified seed in international trade. The National Designated Authority responsible in each country for the operation of the OECD Seed Schemes is required to use the methods outlined or to adapt them where local conditions make this necessary; such adaptations should, however, conform to the general principles established in the present Guidelines.

# Part I.

# CONTROL PLOT TESTS

## Part I. CONTROL PLOT TESTS

### *Purpose*

8. Control plot tests are used to monitor the identity and purity of variety (being hybrid or non-hybrid), at various stages in the seed multiplication programme, thereby assuring the Designated Authority that the quality of seed produced in the OECD Schemes is of a satisfactory level.

9. The control plot tests are designed to answer two questions:

(a) Does the sample generally conform to the description of the variety, thereby confirming its identity ;

(b) Does the sample conform to the published standards for varietal purity.

10. The first question can be answered by making a visual comparison between the control plot, sown with a sample of seed representative of t he seed lot, and a plot grown from a reference sample, hereafter referred to as the "Standard Sample".

11. The second question requires the identification of off-type plants within the control plot so that their numbers can be related to the standards published in the OECD Seed Schemes. This test measures the uniformity of the seed lot and determines whether or not the characteristics of the variety have remained unchanged during seed multiplication; it will also indicate the effectiveness of limiting the number of crop generations.

### *Pre-control*

12. When a seed lot is being multiplied to produce a further generation of seed, the information provided by a control plot is invaluable in that it gives the Designated Authority data on identity and quality which are available before --or about the same time-- as the next seed crop is ready for field inspection. In this instance the test, which is referred to as a pre-control test, is grown simultaneously with the seed crop of the following generation. It provides important and essential information which is additional to that obtained at the seed crop inspection and becomes an integral part of the process of certifying seed.

13. There should be conformity between the pre-control plot results and those from the field inspection. If the results do not conform, the evidence must be carefully examined and the decision to reject will depend on the circumstances; the off-type characteristic may be such that it can be seen in the plot, but is less easily observed in the seed crop (e.g. a transient characteristic which can be seen only at a particular stage of development of the plant). In some rare instances it may be evident that the control plot result is not representative of the seed lot as it appears in the seed crop suggesting that the sampling procedure is at fault. Differences between the field inspection and the control plot could also result if herbicides have been used causing distortion of plant characters in the field, if volunteer plants are present in the seed crop or if the seed producer has reduced the number of off-types in the field by roguing.

14. Although field inspections are an essential requirement of OECD Seed Schemes, there are many advantages available to Designated Authorities in conducting Pre-control plots. These are as follows:

(a) Plants representing the seed lot of the variety can be observed as frequently as is necessary.

(b) The observation period can be extended from seedling emergence to full maturity.

(c) All plants in the control plot population can be examined in detail if necessary.

(d) A comparison can be made with the Standard Sample.

(e) Comparisons can also be made with seed lots of the same variety in the same and previous generations.

(f) One expert can make judgements on all control plots for all varieties and categories thus ensuring the standardisation of recording.

(g) Where the land is free from volunteers and clean machines have been used for sowing, the Designated Authority can be certain that all off-type plants observed in the control plot have arisen from the seed sample.

(h) Designated Authorities may use an adverse pre-control plot test result to reject seed crops sown with the same seed lot.

***Post-control***

15. When a control plot is grown as a post-control test to monitor the quality of the seed produced, the results are not usually available until the end of the next growing season after the seed was harvested.

16. Very often the seed lot to which the post-control relates was used to sow either another seed crop or a commercial food or industrial crop, and the test result will come too late for remedial action unless the seed lot --or parts of it-- was not commercialised. These post-control tests are nevertheless valuable because they monitor how efficient or not the seed production process has been in maintaining varietal purity and identifying ways in which the system might be improved. By allowing comparisons between plants grown from the seed lot produced and those grown from the Standard Sample, the Designated Authority can monitor quality and give assurance that the minimum standards are being upheld.

17. For seed to be further multiplied, one control plot can serve two functions: that of post-controlling a seed lot from the last harvest and pre-controlling a seed crop for the next harvest.

18. In the case of hybrid varieties, because the varietal identity and purity of the hybrid cannot be verified in the seed production field, it is necessary to assure production quality in the post-control plots.

19. The hybrid variety observed in post-control plots must be true to a variety, and the plants must conform to the characteristics of the hybrid listed by the Designated Authority at the time of the Registration.

20. The parental components for the production of the hybrid variety must be observed in the pre-control plots.

***Off-season control***

21. In order to obtain results from observations in control plots without waiting for the end of the following growing season, it is possible to conduct these tests (pre-control and post-control) in a region of another hemisphere. The quality of the basic seed, of hybrid parental components, and of non-commercialised seed in stock can thus be determined before the following sowing period.

***Standard Sample***

22. The checking of varietal identity and purity in pre-and post-control can be done best by comparing plants grown from a sample of the seed lot with plants grown from seed of the variety "Standard Sample".

23. The purpose of the Standard Sample is to provide a living description of the variety; its supply, maintenance and authentication are critical.

24. It is important to take into account that there are often two official reference samples held by the authorities responsible for Registration and Certification.

25. The first is the sample which is used by the Authority in charge on the National List of Varieties. When a new variety is submitted for registration, the Listing Authority uses a sample as the official standard in tests for assessing distinctness, homogeneity and stability (hereafter called "Definitive Sample"). It is the Listing Authority which retains and uses the Definitive Sample primarily for Registration purposes. The sample should be sufficiently large to satisfy requests for small quantities of seed both from within the participant country and also from other Listing Authorities. In some cases it may be difficult to satisfy requests for seed from Designated Authorities for certification purposes, since the quantities required may be large and therefore would use up the Definitive Sample too quickly.

26. The second reference sample is the Standard Sample. It is used by the Designated Authority as the official standard in pre-and post-control plots against which all other samples of seed of the variety in seed certification are judged for trueness to variety. It is the Designated Authority which retains and uses the Standard Sample of listed varieties specifically for use in seed certification. Before use, the Standard Sample should be checked and verified by both the Listing Authority and the Designated Authority to ensure that it is authentic and identical to the Definitive Sample.

27. The Standard Sample should be obtained by the Designated Authority direct from the breeder or the maintainer. Alternatively, where relatively large amounts of seed of the Standard Sample are required, not only for the annual establishment of control plot tests of certified seed, but also to meet requests for seed from other Designated Authorities, it is permitted to use a sample from a Pre-basic seed lot which has been checked for uniformity and trueness to variety against the Definitive Sample.

28. In the case of synthetic varieties of allogamous species and all hybrid cultivars, it is the final generation of Certified seed which will constitute the Standard Sample. For certain species and for hybrid varieties it may be necessary to have separate Standard Samples which represent the inbred lines and parental components which are used at the Basic and Pre-basic seed level to produce the hybrid cultivar.

29. Some countries may use the Definitive Sample in place of the Standard Sample for seed control plot tests and in these cases the Definitive Sample has a dual function. However, this will only be practicable where the demands for certification control purposes are small.

30. In the case of Standard Samples of varieties originating from the country of another Designated Authority it is essential for the Standard Sample to be obtained from that Authority and not directly from the breeder.

31. The Standard Sample which is recognised as truly providing a living description of the variety during the test period is the most reliable standard by which seed certification samples can be judged. It should be used in conjunction with the official description bearing in mind that the description of varieties may have some limitations since it is not always sufficiently precise for the purpose of classifying and identifying varieties.

32. When the germination of the Standard Sample begins to fall or the stock of seed needs replenishing, a new sample should be requested. There must, however, be sufficient time allowed for the comparison of the new sample and the old sample in a field test for at least one cropping season in order to check its authenticity and before the original Standard Sample is discarded.

***Previous cropping***

33. In siting control plots, the Designated Authority or its agent must take care to ensure that the field is suitable. There must be no risk of contamination from volunteer plants of the same or closely related species or similar crop groups. This is done by checking the previous cropping of the field to be used and ensuring that a carefully planned rotation has allowed the field to be cleaned after harvest of seeds shed by both crop plants and weed species.

34. Seeds which remain dormant or which are prevented from germinating before being cultivated into the soil can present special problems. The seed of a number of species of crop plants can survive in the soil for a number of years if the conditions are suitable. Seeds with a high oil content such as those of rape (*Brassica napus*) and turnip rape (*Brassica rapa*) are known to remain viable for many years, but there is also evidence that seeds of small grained cereals can also survive for several years when conditions are favourable.

***Control plot layout***

35. The control plot tests should be designed in such a way that observations can be easily made.

36. A simple layout with all samples of the same variety grouped together will provide the best basis for comparison with the Standard Sample included for reference (this is also true for the hybrid variety components). It is also an advantage to put similar varieties in close proximity to highlight whatever slight differences exist between them.

37. Within a variety, recording is facilitated if related seed lots which share the same antecedent are sown in neighbouring plots. In this way contaminants seen in one plot can be readily examined for their presence in another.

38. The plots should be duplicated in another part of the field whenever possible and if resources permit, so that additional data can be obtained. For some categories of seed it may be essential to replicate in order to achieve the minimum number of plants for recording. When characteristics have to be measured, a more formal design is required such as a randomised block layout. For some species of grasses and fodder legumes it may be necessary to use a spaced plant design for the control plots to allow measurements of morphological characters such as leaf length, leaf width, plant height etc. to be made on single plants.

39. The design of the test should be such as to enable appropriate statistical analysis of the results and a decision to be reached on the basis of conventional confidence limits.

40. In the determination of varietal purity, the standards are expressed either as a percentage of the population or where plant populations are not easily determined as a number per unit area. The off-type plant count in the plot can be used to give a probability of the seed lot meeting the published standards provided the plot size is sufficiently large. Reject numbers should be used which relate the number of off-type plants observed in a sample to a published standard in such a way that reasonable account is taken of the risks of incorrect acceptance or rejection of the seed lot. The degree of risk is related to the sample size.

***Husbandry***

41. A good uniform seed bed is desirable to promote the rapid and uniform establishment of control plots.

42. The husbandry requirements for control plots are usually similar to those for commercial crops with the exceptions that variety differences and characteristics should be maintained whenever possible and the condition of the plots should permit examination throughout all the relevant growth stages. It may be necessary to keep fertiliser levels at a minimum in order to avoid lodging, especially in cereal crops.

43. Care should also be taken in the use of herbicides and plant growth regulators which could affect the morphology of the plant.

***Recording***

44. Recording of the control plots should start when plants reach growth stages at which varietal characteristics can be observed. According to the species this can be during vegetative growth stages, at flowering or at full maturity. Control plots can also be recorded for species purity and for the presence of seed-borne diseases.

45. The main characteristics which may be used in control plot tests is included in the latter part of the document. For many species it is based on the characters included in the UPOV Guidelines for the Conduct of Tests for Distinctness, Homogeneity and Stability, and is divided into "primary" and "secondary" characteristics. It is recommended that primary characteristics, which are usually obligatory for the purposes of UPOV descriptions, are assessed in the case of OECD control plots.

46. Recording varietal or species purity --or levels of seed-borne disease-- involves the identification of plants which are different in appearance. Plants which are atypical for major plant characters should be examined in more detail. A method of recording the individuals and identifying them is required so that they are not counted again on future visits; labels, markers or coloured wool have been used successfully for this purpose.

47. The average plant population of each control plot should be estimated in order to be able to calculate the level of impurities present. Where the number of variants in a plot is close to or in excess of the likely reject value the plant population should be more accurately assessed.

48. Although it is usually possible to see when a plot has the wrong identity or is badly contaminated with an off-type plant it may, however, be difficult to decide whether or not an individual plant should be classed as an off-type. Such decisions require the experience of a plot recorder who is an expert in the examination of the morphological characteristics of the species under study and also has detailed knowledge of the characteristics of the variety.

49. "Subjective" judgements must be made with the help of the variety description, as to whether the off-type is a genetic variant or whether a normal variation between plants has been exaggerated by environmental factors. In general terms, the recorder should ignore small variations and only include clearly distinct off-types in the final count which may determine acceptance or rejection of the sample.

50. In plots where the number of off-type plants can be counted in relation to the total number of plants in the population, and if population is sufficiently large, then it is possible to calculate a probability of the seed lot being of the required level of varietal purity.

51. In the case of male sterile hybrid component, in addition to the varietal purity assessment, all the plants of the plot should be carefully checked to determine if any are producing viable pollen.

52. *Particular case of hybrid rye (Secale cereale).* Hybrid rye seed production involves the physical mixing of a restoring pollinator with a single-cross hybrid. The sample drawn from the resulting three-way cross hybrid production is consequently a mixture of the hybrid seeds and of self-pollinated pollinator seeds. This must be considered when counting impurities in the post-control plots.

***Laboratory tests***

53. In addition to the examination of plants in control plots there are a few laboratory seed tests which can be used for identifying the varieties of some species.

54. A first classification can sometimes be made from a visual observation of seed characters such as shape, size, colour ornamentation or other physical features. Generally, this will identify not only the species but also a classification group and may even identify individual seeds which are admixtures in the sample.

55. Germinated seeds can often show additional characteristics as for example in the presence or absence of anthocyanin pigmentation in the coleoptile of rye (*Secale cereale*).

56. In some species the ploidy level can be used to classify varieties, for example diploid and tetraploid ryegrass (*Lolium perenne*).

57. A second classification may be possible in some species using chemical tests such as electrophoresis. In others such as rape (*Brassica napus*), tests for erucic acid and glucosinolate content can be used; for wheat (*Triticum aestivum*) the reaction of seeds to phenol has been used successfully to identify seeds which are possibly variants.

58. Some Designated Authorities also examine seeds of barley (*Hordeum vulgare*) for morphological characters which have been used in the classification and description of a variety but which can only be observed under the microscope.

59. The use of electrophoresis and other chemotaxanomic techniques have been used successfully to identify varieties in some crop species. This technique has proven to be useful for the purposes of Registration where the number of seeds which need to be tested for a particular variety is relatively small. It seems unlikely, however, that electrophoresis will be used large-scale in seed certification for determining varietal purity because of the high costs involved in testing large numbers of seeds for each seed lot. Electrophoresis may be used for confirming the identity of small numbers of individual seeds where other tests have been inconclusive.

60. In the case of hybrid varieties of sunflower *(Helianthus annuus)*, rape (*Brassica napus*) or maize *(Zea mays)*, it is possible to estimate the level of hybridity by using electrophoresis. The varietal purity of these species hybrids can also be estimated with this technique.

***Reject numbers***

61. The concept of “reject numbers” is to relate the number of off-type plants observed in a sample to a published standard in such a way that reasonable account is taken of the risks of incorrect acceptance of rejection of the seed lot.

62. A set of “reject tables” is used rather than a straightforward application of the standard. The standards are converted into reject values using the binomial probability distribution. A sample is considered to be non-conforming to the standard -- and rejected-- if the number of off-type plants is equal to or greater than the reject number for a given population.

63. In choosing a sample size, the costs and time involved in observing large samples have to be balanced against the risks of reaching a wrong decision. As a general rule, a sample size of 4 x n can be used when the standard level to be applied is 1 for n.

64. Table 1 below provides the reject numbers for various sample sizes and standards.

*Example: For a varietal purity standard of 99.9%, i.e. an impurities threshold of 1 per 1000, the rejection rule [i.e. 9 or more off-type plants out of a sample of 4000 plants observed] limits the risk of incorrectly rejecting a seed lot to 5 % ($\alpha < 0.05$).*

Note: This system is biased in favour of the seed producer, since the risk of an incorrect acceptance of a seed lot is higher than the risk of an incorrect rejection.

65. Reject numbers above the lines in Table 1 (white background) are not as reliable as those below (grey background), because the sample size is insufficiently large and there is a greater risk of an incorrect acceptance of unsatisfactory seed lots.

**Table 1. :** Reject numbers for various sample sizes and varietal purity standards ($\alpha < 0.05$)

| **Sample size** ***(plants/ears)*** | **Varietal purity standard** | | |
|---|---|---|---|
| | **99.9%** | **99.7%** | **99.0%** |
| | **Reject number** | | |
| 200 | -- | -- | 6 |
| 300 | -- | -- | 7 |
| 400 | -- | 4 | 8 |
| 1000 | 4 | 7 | 16 |
| 1400 | 5 | 9 | 21 |
| 2000 | 6 | 11 | 29 |
| 4000 | 9 | 19 | 52 |

Note: The symbol "--" indicates that the sample size is too small for a valid test of the sample.

# Part II.

# FIELD INSPECTION OF SEED CROPS

## Part II. FIELD INSPECTION OF SEED CROPS

### *Purpose*

66. The field inspection of growing seed crops is the second procedure required by the OECD Seed Schemes. The most important functions are to check that the seed crop shows the characteristics of the variety which it claims to be (**varietal identity**) and to ensure there are no circumstances which might be prejudicial to the quality of the seed to be harvested (**varietal purity**).

67. Seed crops may be inspected frequently during the growing season. There must be at least one inspection which is timed to allow the best opportunity to assess varietal identity and purity, but there may be more.

68. With many crops the ideal time for field inspections to be carried out is during the flowering period or immediately before dehiscence of the anthers. With some crops a vegetative inspection is also required and with others, observations at full maturity are essential.

69. Although the technique of field inspection differs in detail depending on the particular features of each species, the main principles for checking at field inspection are as follows:

(a) The previous cropping history of the field should be such that the risk of undesirable volunteer plants of the same or related species contaminating the seed crop is reduced to a minimum.

(b) The seed crop should be sufficiently isolated from other crops to reduce the risk of contamination with undesirable pollen.

(c) The crop should be physically isolated to prevent mechanical admixture at harvest.

(d) The seed crop should be isolated from sources of seed-borne disease.

(e) The seed crop should be reasonably free from weeds and other crop species, especially those whose seeds may be difficult to separate from the seed crop during seed processing.

(f) The seed crop should be free from seed-borne diseases.

(g) The seed crop should have the correct varietal identity.

(h) There should not be more off-type plants present than the varietal purity standards allow.

(i) There should not be more plants of other species present than the standards allow.

(j) For hybrid varieties the proportion of male to female plants should be satisfactory and as defined by the maintainer. The physical or genetic emasculation of female seed-bearing plants should be effective.

***Principles***

70. The person conducting the field inspection should be provided with all information about the seed crop. The inspector should be an expert in recognising the characteristics of the species which are used for distinguishing varieties, and have a sound knowledge of the varieties to be inspected. The information provided should include a description of the variety --or of the parental lines/components in the case of hybrid production. He should also be informed of the history of the seed used to sow the seed crop, together with results from the pre-control plot grown concurrently by the Designated Authority. The cropping history of the field for the past five years should also be available to the inspector.

71. The inspector is required to give an independent opinion of the seed crop and is responsible to the Designated Authority. The function of the inspector is to report the state of the crop at the time of inspection. The timing of the inspection may be such that some off-types may be hidden or difficult to identify, in which case a second or subsequent inspection might be required before a decision can be reached.

72. The inspection of the seed crop should be supplemented by results from the pre-control plot, which the Designated Authority has under continuous observation, and which will provide the inspector with reliable data on all varietal identity and purity aspects relevant to the seed lot(s) used.

***Previous cropping***

73. The crop inspector should interview the grower of the seed crop concerning details of the previous cropping of the field. The grower should provide details relating to the crops grown on the field for the previous five years. Information about possible sub-division of a field in previous years, or any previous cropping with the same variety, can also be established at this time.

74. In the case of hybrid production, the same field cannot be used consecutively for the same species, to avoid the growth of fertile volunteers from hybrid seed production of previous years.

***Authentication***

75. In order to authenticate the identity of the seed sown, growers should retain at least one label from each seed lot used to sow the crop. The grower must also display a second label of each seed lot used in the field, to be clearly visible to the inspector.

76. For hybrids, labels of the seed lots used for male parent and for female parent must be kept and verified.

77. The purpose of this procedure is to check the details provided on the label against those on the crop inspection form, and to confirm the identity of the variety.

***Varietal identity***

78. The first function of the field inspection is to examine the seed crop as a whole to ensure it is consistent with the characteristics of the variety given in the official description. This is usually done by walking into the seed crop and examining a reasonable number of plants. The actual number to be examined in each case will depend on the complexity of the distinguishing characters and the uniformity of the variety. Thus it would be necessary to examine a larger number of plants for allogamous species than for autogamous species.

79. For some species, positive confirmation of identity of individual varieties may not always be possible in the seed crop, but it should always be possible to ensure that the crop is in the correct variety group. Access to the control plots will allow the inspector to become familiar with the characteristics of the variety and be aware of the differences with other varieties in the same variety group.

80. In the case of hybrid varieties, the inspector must be able to identify without difficulty the male parental line and the female parental line. He must check the varietal identity of each parental component using the corresponding official descriptions.

***Condition of the seed crop***

81. After having examined the field as a whole, the inspector should examine the field in more detail, especially around the perimeter.

82. Observations should be made for signs which would indicate that part of the field might have been sown with different seed or might have become contaminated, for instance, in field gateways or on headlands. Places in the field where sowing started should be located to check that the drilling equipment used to sow the crop had been properly cleaned before use. Particular attention should also be given to the presence of other crop species, weeds, seed-borne diseases, and verification of isolation from sources of contaminating pollen.

83. The general appraisal of the seed crop should determine whether or not it is in satisfactory condition to permit the detailed examination of plants for varietal purity.

84. Crops which are severely lodged, badly infested with weeds, stunted or poorly grown because of disease, pests or other causes and which cannot be assessed for varietal purity should be rejected. The inspector, however, could use the evidence of the pre-control plot to supplement the information in the field in intermediate cases.

***Isolation***

85. Isolation of the seed crop should be checked whilst walking around its perimeter. For crop species which are cross-pollinated by insects or wind, this will involve checking all surrounding fields for any crops lying within the minimum prescribed isolation distances which might cross-pollinate with the seed crop.

86. Where the isolation distance between the hybrid seed crop and a source of contaminating pollen is insufficient to satisfy the minimum requirements, the inspector must request partial or total destruction of the contaminating source so that the desired isolation distance is met.

87. When isolation is satisfied by the existence of a pollen barrier of the male parent of the hybrid around the crop to produce the hybrid seed, the inspector must be assured of the coincidence of flowering between the male and female parents.

88. The minimum isolation distances are given in the OECD Seed Schemes.

89. A map of the seed crop and the surrounding crops, provided by the grower, should alert the inspector to potential sources of foreign pollen.

90. The inspector should also look for volunteer plants or weeds, both in the seed crop and neighbouring crops, which could also be a source of contaminating pollen. In the case of seed production of hybrid *Sorghum spp*, he should look for any potential plants of other sorghum species (in particular Aleppo Grass *Sorghum halepense*).

91. Additionally, seed crops of autogamous species and apomictic varieties of smooth-stalked meadowgrass (*Poa pratensis*) should be isolated from other crops by a definite barrier or a space sufficient to prevent physical admixture during harvesting.

92. Checks should also be made to ensure that seed crops are isolated from other crops which may be infected with seed-borne diseases.

***Species purity***

93. For many crop species, the rules of the Schemes do not include species purity standards to be applied in the seed crops.

94. Nevertheless, for some crop species, there are minimum species purity standards in addition to those for varietal purity and these must be assessed at the time of crop inspection.

- Grass and Legume Seed Scheme: see Appendix 2, section 7 for *Lolium* and for other species;

- Seed Scheme for Crucifer and Other Oil or Fibre Species: see Appendix 2, section 7;

- Maize and Sorghum Seed Scheme: for non hybrid varieties of *Sorghum bicolor* and *Sorghum sudanese* see Appendix 2, section 6 -- for *Sorghum* spp. varieties, see Appendix 2, section 10.

95. In the case of seed crops that produce hybrid varieties of *Sorghum* spp., they shall contain not more than 1 plant in 10 $m^2$ of another species of *Sorghum*, the seeds of which are difficult to distinguish in a laboratory test from the crop seeds or which will readily cross-pollinate with the crop being grown for seed.

96. If there are varietal impurities in addition to plants of other species, the inspector should add these together and apply the appropriate varietal purity standard only, the procedures for which are given below.

97. Nevertheless, the presence of a number of crop species and weed species in a seed field can create problems not only in the seed crop but also in the processing of the seed.

98. In particular, the following are recognised as causing difficulties in some countries:

- ryegrass (*Lolium* spp) and cereals in seed crops of other cereals species;
- field pea (*Pisum sativum*), field bean (*Vicia faba*) and french bean (*Phaseolus vulgaris*) in seed crops of lentil (*Lens culinaris*) and chickpea (*Cicer arietinum*);
- cow pea (*Vigna* spp) in field pea (*Pisum sativum*).

99. There are also a number of weed species which can prove difficult to clean from seed during seed processing. The following associations have been identified in various countries:

- ryegrass (*Lolium remotum*) in linseed (*Linum usitatissimum*);
- wild mustard (*Sinapis arvenis*) in white mustard (*Sinapis alba*), rape (*Brassica napus*), turnip rape (*Brassica rapa*) and brown mustard (*Brassica juncea*);
- Aleppo sorghum (*Sorghum halepensis*) in hybrid Sorghum;
- oat grass (*Avena fatua*), wheatgrass (*Agropyron repens*), wild radish *(Raphanus raphanistrum*), (*Gallium* spp), (*Bromus secalinus*) and cockle (*Lolium temulentum*) in cereals.

***Varietal purity: Requirements for all crops***

100. Assuming crop location, authenticity, varietal identity, isolation and crop condition are all satisfactory, the final stage in the inspection is the assessment of varietal purity.

101. To do this it is necessary to follow a sampling procedure which will focus attention on small areas of the seed crop for detailed examination.

102. The number and size of these areas have to be related to the specific minimum varietal purity standards appropriate to the crop species and the category of seed being produced.

103. In deciding how many sample areas should be examined it is necessary to balance the requirements for statistical accuracy and the need for reasonable confidence in the result on the one hand, against the time available for making inspections on the other hand. This may involve a compromise in favour of reducing the workload for practical reasons and, as a result, the risk of reaching the wrong decision is increased. Generally, there is a bias in favour of accepting a crop which may have an impurity level greater than the desired standard, but this can be justified since the standards set for varietal purity are normally higher than is strictly necessary for commercial crop production.

104. The location of the sampling areas should be such that the whole field is effectively covered and means that the inspector should work by a pre-determined procedure. This, however, may have to be adapted to the shape and size of each field, to the particular features of each species, but in particular whether the standard for varietal purity is expressed as a percentage or as a maximum number of off-types per unit area.

105. The distribution of the sampling areas should be random and widespread so as to represent the whole crop and there should be no conscious selection of areas which appear to be any better or worse than the average for the crop. This can be achieved in practice by deciding on a pre-determined distance between each sample area but should also take account of the direction of drilling so that each sampling area should attempt to include a different pass of the seed drill.

106. The 3rd part of the present document "*Characteristics for assessing varietal purity*" provide for each species those morphological and physiological characters which have been found to be of greatest use in distinguishing between varieties, thus finding the off-type plants (varietal impurities). The varietal impurities may include other identifiable varieties, deviant plants or various varietal types.

107. The impurities in the seed crop will differ considerably in the ease with which they can be observed. Differences such as height, colour, shape, maturity are clearly identified but less obvious impurities for example leaf shape, leaf hairiness, flower and seed characters may only be detected by examining a particular part of the plant. Larger samples can be examined for obvious impurities than for those which are less obvious and these should be taken at random and from as wide an area of the field as possible.

108. The results of the relevant control plot corresponding to the basic seed lots used should be made available to the inspector. This will enable off-types found in the pre-control plot by the Designated Authority to be positively confirmed in the seed crop. There may also be off-types which are present in the seed crop but which were not observed as occurring in the control plot; these too need to be recorded and taken into account when determining whether the crop is acceptable or not.

***Varietal purity: Additional requirements for hybrid crops***

109. When inspecting crops to produce hybrid varieties, the inspector must be assured, before verifying the varietal purity of the male and female components, that there has been no accidental mixing of the two component rows.

110. In the case of maize, sorghum and sunflower hybrid seed production, purification by roguing is an acceptable method for obtaining varietal purity for one or other of the two parents. In this case, the removal of plants aberrant for one or several characteristics must be done before any pollen is shed.

111. In the case of using the male sterility, the inspector must be assured of the absence of male fertile or partially male fertile plants in the female parent rows.

112. In the case of mechanical emasculation for the production of maize hybrid seeds, the inspector must be assured that it is applied before the female plants have shed any pollen, and above all before the stigmas of the female plants are receptive.

113. During the field visits, the inspector must be informed by the seed grower of the harvesting conditions to ensure there will not be any risk of mixing between the male parent and the female parent. The rows of the male parent will be harvested separately and before those of the female parent. This does not concern production of hybrids of rye (*Secale cereale*) or rape (*Brassica napus*) where the male and female lines are cultivated as mixed.

114. The varietal purity of the hybrid obtained in the production crop can only be checked in a post-control plot sown with a sample of the hybrid seed produced. However, the varietal purity can be secured by ensuring that the following requirements are met:

(a) adequate isolation distances from sources of contaminating pollen;
(b) good conditions for the pollen dissemination;
(c) high levels of male sterility of the female parent;
(d) low levels of sibling;
(e) high levels of varietal purity of both parents;
(f) separate harvesting of the male component ahead of the seed-bearing (female) parent.

***Inspection of conformity to percentage standards***

***-- Impurities counting***

115. For standards expressed as a percentage, the number of impurities observed in the sample areas has to be related to the plant population.

116. An estimate of the plant population can be obtained by counting the number of plants or ear-bearing tillers in a row of 1 metre length, but in the case of broadcast crops in 0.5 $m^2$ areas.

117. The population per hectare for crops in rows can be calculated using the following formula:

$$P = \frac{1{,}000{,}000\ M}{W}$$

where P = the plant population per hectare
M = the mean number of plants per metre length of row
W = the width between rows (in centimetres)

For some crops such as cereals it is usually quicker to count fertile tillers in the form of ears or panicles rather than plants. An assumption then has to be made that each single plant will on average produce the same number of ears so that the counts obtained are proportional. The value of M is obtained by counting the number of plants or ear/panicles in a row of 1m length within each sample area and taking the mean.

118. The population per hectare for broadcast crops can be calculated using the following formula:

$$P = 20{,}000 \times N$$

where P = the plant population per hectare
N = the mean number of plants per 0.5 $m^2$

The value of N is obtained by counting the number of plants or ears/panicles in an area of 0.5$m^2$ within each sample area and taking the mean.

***-- Sampling areas***

119. The size and number of sampling areas will vary depending on the species to be inspected, the size of the field, whether the crop is drilled or broadcast, whether it is self or cross-pollinating and the geographical area in which the crop is being grown. In practice, the Designated Authority will determine the appropriate size and number of sampling areas for each crop to guarantee that sufficient plants are examined in order to apply minimum standards for varietal purity.

120. For cereals, ten sampling areas each of 20 $m^2$ and containing an average of 500 ear-bearing tillers per $m^2$, would give a total sampling population of 100,000. For other crop species this model should be followed wherever possible but may have to be adapted according to local circumstances.

121. In the case of crops sown in wide rows, the size of each sample could be a 20-25 m length of row including the space between the rows. Thus for maize, sorghum and sunflower, some Designated Authorities might consider a total of 1,000 plants to be a sufficiently large sample, while for soybean the number could be 3,000 to 10,000 depending on the category to be inspected.

122. Where crops are broadcast it may be possible to reduce the size of each sample area to ensure that the total number of plants examined is not more than is required statistically to give a good estimate of varietal purity.

123. In general, the number of sampling areas should increase in proportion to the size of the field. Because of higher standards for Pre-Basic and Basic Seed crops, the number of plants examined in these higher category crops should also be larger than for Certified Seed.

124. As a general rule, a sample size of 4 x $\underline{n}$ can be used when the impurities threshold is 1 for $\underline{n}$; thus for a minimum varietal purity of 99.9 per cent (1 in 1000) the sample size should be 4000.

125. In some crops grown for hybrid seed production, it is essential to examine all plants in the sample and to check not only for varietal purity but also that the standard for male sterility of the seed-bearing parent has been achieved.

126. For some crop species, there may be important distinguishing characters, which are described in the official description, but which are too small to be examined under field conditions. These characters could be critical in the assessment of uniformity of a variety and could indicate out-pollination, segregation or mutation in the seed lot. In such circumstances, plants could be examined more easily under laboratory conditions.

127. The Designated Authority can rely mainly on the data from the pre-control plot and use the field inspection results for confirmation only. Where there is an obvious discrepancy between the control plot and the field data, it may be necessary to conduct further examinations in both areas so that a positive decision can be reached.

-- ***Reject numbers***

128. For assessing crops against the standards, 'reject numbers' as described in the previous section relating to Control Plot Tests can be utilised (*see paragraphs 61 to 65*).

129. Some examples are given in Tables 2 and 3 for a range of standards, populations and corresponding reject numbers which might be encountered in a total sampling area of 200 square metres (10 x 20 $m^2$).

**Table 2.:** Reject numbers for a total sample area of 200 $m^2$ for various varietal purity standards *(99.5 to 99.9%)*

| **Estimated population (plants/ears per ha)** | **Varietal purity standard** | | |
|---|---|---|---|
| | **99.9%** | **99.7%** | **99.5%** |
| | **Reject number for a sample area of 200$m^2$ (*)** | | |
| 600,000 | 19 | 47 | 74 |
| 900,000 | 26 | 67 | 107 |
| 1,200,000 | 33 | 87 | 139 |
| 1,500,000 | 40 | 107 | 171 |
| 1,800,000 | 47 | 126 | 203 |
| 2,100,000 | 54 | 146 | 235 |
| 2,400,000 | 61 | 165 | 267 |
| 2,700,000 | 67 | 184 | 298 |
| 3,000,000 | 74 | 203 | 330 |
| 3,300,000 | 81 | 222 | 361 |
| 3,600,000 | 87 | 241 | 392 |
| 3,900,000 | 94 | 260 | 424 |

**Table 3.:** Reject numbers for a total sample area of 200 $m^2$ for various varietal purity standards *(97.0 to 99.0%)*

| **Estimated population (plants/ears per ha)** | **Varietal purity standard** | | |
|---|---|---|---|
| | **99.0%** | **98.0%** | **97.0%** |
| | **Reject number for a sample area of 200$m^2$ (*)** | | |
| 200,000 | 52 | 96 | 139 |
| 400,000 | 96 | 182 | 266 |
| 600,000 | 139 | 267 | 392 |
| 800,000 ** | 182 | 350 | 517 |

(*) Crops are rejected if the total number of impurities found in the total area of 200$m^2$ is equal to or greater than the number given for the appropriate estimated population and varietal purity standard.

(**) With varietal purity levels of 99.0% and below and high plant populations in excess of 1,000,000 per ha, it is not necessary to use reject numbers. This is because the number of impurities which need to be counted to effect a rejection of the crop are so large that the difference between the expected number and the reject number is small enough to be ignored for practical purposes.

130. Species purity and varietal purity should be assessed separately and both must be satisfactory for the crop to be accepted.

***Inspection of conformity to maximum number per unit area standards***

131. For many crop species it is neither possible nor practical to accurately estimate plant populations because of the cropping system applied; in these cases varietal purity standards are expressed as a maximum number of impurities per unit area (see the OECD Seed Schemes).

132. Following an inspection of the whole field --to check isolation conditions, limited presence of weeds, varietal identity and crop homogeneity-- then one of the following sampling methods A or B described below should be used.

133. The sampling procedures are based on the following assumptions: off-type plants and plants of other species are randomly distributed throughout the crop, and the counts of impurities follow the Poisson distribution.

134. If there are patches of impurities in some parts of the field then the above assumptions become invalid. In such cases these patches should be excluded from the sampling areas and inspected separately.

135. In the design of sampling procedures when inspecting a maximum number of impurities per area standards, *the risk of making a wrong decision is biased in favour of the seed grower,* with a lower risk of the crop being wrongly rejected ($\alpha$) and a greater risk that the crop might be wrongly accepted ($\beta$). This bias in favour of the seed grower can be compared to the case of crops inspected to percentage standards.

136. The two methods described below have been designed to check the conformity of the seed crop to a maximum threshold of 1 impurity per 10 $m^2$. Both methods accept a *20% $\beta$ risk* of accepting fields for which the true level of off-type plants (or plants of other species) is 1.5 in 10 $m^2$, and a *less than 10 % $\alpha$ risk* of rejecting fields with a maximum of 1.0 impurity in 10$m^2$.

137. The two methods of sampling differ in that method A allows for a maximum of 2 successive sets of counts (double plan); method B is based on a sequential sampling technique with a maximum of 9 successive sets.

***-- Method A / Double plan***

138. This method allows for a maximum of two steps. The field size is limited to 10 hectares with counts for impurities being made in 11 sample areas each of 10 $m^2$. Where the seed crop is larger than 10 ha, the field should be sub-divided into two parts with each being inspected separately.

139. If the total number of impurities is equal to or less than 11, the field is considered to have met the minimum varietal purity standard of 1 impurity per 10 $m^2$; if the total is equal to or greater than 18, the standard is exceeded and the field should be rejected.

140. When the total number of impurities falls between 12 and 17, the method requires 17 additional counts to be made. If the new total of impurities obtained for the 28 sample areas is equal to or less than 35, the standard is met and the field can be approved; if it is equal to or exceeds 36, the field is rejected. The exact risks associated to this procedure are $\alpha = 0.086$ and $\beta = 0.198$.

-- ***Method B / Sequential sampling***

141. This method is a sequential sampling scheme in which the number of sample areas inspected is not predetermined but depends on the results of successive sampling.

142. Method B has been designed to save time, but this gain is only effective in practice when a majority of crops meets the varietal purity standard for certified seed of 1 impurity in 10 $m^2$. As in Method A, the field size is limited to 10 ha. For fields in excess of 10 ha the seed crop should be sub-divided with each part inspected separately.

143. The minimum number of counts which should be made is determined by the size of the field (Table 4).

**Table 4.:** Minimum number of counts for various sizes of field

| Size of field (ha) | Number of counts |
|---|---|
| 1 or 2 | 4 |
| 3 or 4 | 8 |
| 5 to 7 | 12 |
| 8 to 10 | 16 |

144. The total number of impurities in the counts is then assessed against the criteria for acceptance or rejection (see Table 5).

**Table 5.:** Acceptance and rejection limits for stated numbers of counts

| Number of counts | Total number of impurities | |
|---|---|---|
| | **FIELD ACCEPTED if number is equal to or less than** | **FIELD REJECTED if number is equal to or greater than** |
| 4 | 1 | 10 |
| 8 | 6 | 15 |
| 12 | 12 | 19 |
| 16 | 18 | 24 |
| 20 | 22 | 30 |
| 24 | 27 | 35 |
| 28 | 31 | 39 |
| 32 | 36 | 44 |
| 36 | 43 | 44 |

145. If the number of impurities falls between the acceptance or rejection bands, then further counts should be made (up to a maximum of 36), until a decision can be reached. The exact risks associated to this procedure are $\alpha = 0.096$ and $\beta = 0.202$.