Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

CANADIAN METHODS AND PROCEDURES FOR TESTING SEED (M&P)

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CANADIAN METHODS AND PROCEDURES FOR TESTING SEED (M&P)

PREFACE

The *Canadian Methods and Procedures for Testing Seed* (M&P) are published by the CFIA and provide the "recognized standard methods" required for carrying out "officially recognized tests" as defined in Section 2 of the current Seeds Regulations.

This version of the M&P replaces all previous editions. New editions of the M&P are usually issued annually. This amended edition becomes effective as dated, e.g. Effective 1 July 2020.

Revision table with details of amendments from previous editions.

Previous Version	Previous Version Revision Date	Paragraph revised, deleted, added	Reason for Update
2019 Edition	1st July 2019	Preface	Updated with 2020 Edition Changes
		Section 1.3.2.m and 3.9.7	Revised wording for suspected varietal contaminants
		Section 2.3.5	Clarification of the weights of analysis procedures for crop kinds not listed in the Seed Regulations
		Section 3.5.2.f	Reorganized reporting example for Grade Table XIV (Lawn and turf mixtures)
		Section 3.7.4	Removal of procedure separating multiple seed units when analyzing Dactylis glomerata
		Section 4.11.4	Revised the procedure for calculating pure living seed to using the germination whole number
		Section 4.14.16	Changed split coleoptile in cereals from normal to abnormal
2018 Amended Edition	15th January 2019	Preface	Updated with 2019 Edition Changes
		Section 3.9.8.e	Clarification of identification and reporting for Prohibited noxious weed seeds that are the same Genus but different species
		Section 4.5.2	Clarification of sand particle size requirements for germination testing
		Section 4.9.1	Added statement that the first count can be omitted for tests in sand or soil-less organic media lasting no more than 14 days
		Section 4.14.2	Clarification of the difference between necrosis and physiological necrosis
		Section 4.14.10	Addition to the notes regarding hard seeds for Hairy Vetch, Hungarian Vetch and Common Vetch
2018 Edition	1st July 2018	Preface	Updated with Amended 2018 Interim Edition Changes
		Section 1.1	Added definition for High-value seed
		Section 1.2.e	Added sentence that it is no longer required to notify Seed section when Cannabis species is found in sample
		Section 1.3.2.h and 3.9.3.e	Deleted wording on Cannabis reporting
		Section 1.3.7	New section on returning High-value seed to the customer
		Section 2.1.1	Added a sentence on procedures for sampling High-value seed when the entire lot is smaller than prescribed weight
		Section 2.3.6	New section describing when a reduced test can be performed on High-value seed

		Section 3.4.1.a	Added a sentence below table stating “All other Cannabis spp. must meet minimum purity standards in Grade Table IV
		Section 3.9.2.d	New section on reporting when a reduced test is performed.

The proposal resulting in the changes to this edition were reviewed by staff of the Canadian Food Inspection Agency (CFIA) Seed Science and Technology Section (SSTS), CFIA Seed Section and members of the Commercial Seed Analysts Association of Canada (CSAAC). Input from CSAAC is a valuable contribution to the ongoing revision of this document.

Proposals to amend the M&P must be submitted on the M&P amendment form which is available on the CSAAC website or by e-mailing SSTS at cfia.ssts-ssts.acia@canada.ca.

Any comments or enquiries about the M&P can also be sent to SSTS at cfia.ssts-ssts.acia@canada.ca.

Editor for M&P 2020 Edition: Gord Berg, Science Laboratory Analyst, SSTS

INTRODUCTION

a. PURPOSE

This manual presents the recognized standard methods for testing seed as defined in Section 2, Seeds Regulations. These methods and procedures are to be used to provide the analytical information required to grade a seed lot in accordance with the Seeds Regulations and the Grade Standards. The assessment of any attribute reported on a report of analysis for grading purposes is to be determined from tests conducted on one submitted sample from one sampling operation of a lot. **All seed lots must be free from Prohibited Noxious Weed Seeds listed in the *Weed Seeds Order.*** If a direction in this manual is found to be contradictory to a requirement of the Seeds Regulations, the Regulations take precedence.

These methods and procedures have been developed to meet specific analytical requirements for grading seed according to the Seeds Regulations and the Grade Tables. The definitions, descriptions and procedures of seed analysis used in these methods have, in the main, been adapted from both the Association of Official Seed Analysts' "Rules for Testing Seeds" (AOSA) and the International Seed Testing Association's "International Rules for Seed Testing" (ISTA). The principle differences in the Canadian methods for testing seed are the size of the working sample and the use of a "sequential analysis" system for the determination of numbers of foreign seeds per unit weight. The methods take advantage of the unique Canadian grading system which prescribes the labelling of seed by grade name rather than the labelling of the analytical results.

The end product of these methods and procedures is analytical information regarding the grading factors addressed in the Seeds Regulations and the Grade Tables. These can include, depending on the Grade Table:

(i). The numbers per unit weight (e.g. number per 25 g or per kg) of other crop seeds, weed seeds and inert matter such as ergot/sclerotia;

(ii). The percentages by weight of the components of the seed sample (e.g. percentages of pure seed, other crop kinds, weed seeds, inert matter, etc.);

(iii). The percentages by number of germinable seeds, diseased seeds and seeds of other species.

b. ACCURACY OF THE RESULTS OF SEED ANALYSIS

Subject to the natural variations which occur in random sampling, the accuracy with which the results of seed analyses will represent a lot of seed depends on:

(i). The thoroughness of the blending of the lot of seed from which the sample is drawn;

(ii). The care used in drawing the sample;

(iii). The care with which a number of small samples drawn from several containers are mixed to form a composite sample representing a lot of seed or, when the lot is not homogeneous, the care taken in keeping unlike samples separate;

(iv). The care with which the working sample is taken;

(v). The technical knowledge, skill and accuracy of the analyst;

(vi). The condition of the equipment used.

Of the foregoing factors only (iv), (v) and (vi) are within the control of the laboratory.
For details on sources of variation and uncertainty in seed testing, see the ISTA Position Paper on Quantifying and Reporting uncertainty of Measurement in Seed Testing at www.seedtest.org.

1.0 REPORT OF ANALYSIS

1.1 GENERAL DEFINITIONS

Accredited Analyst: A seed analyst who has completed training in seed analysis and passed the qualifying examination set by the Canadian Food Inspection Agency.

Accredited Test: The test method and procedures conducted are those stated in the current Canadian Methods and Procedures for Testing Seed. The kind or species and the test method is stated on the scope of testing for which the laboratory is accredited.

Amended report of analysis: When a laboratory amends a report as a result of an error on the original report, the amended report issued must state “Amended Report”.

Grade Tables: Refers to the "Tables of Grade Standards" in Schedule I of the Seeds Regulations.

High-value Seed: Describes seed that is not readily available, limited in quantity and/or sold on a per seed basis such as breeder seed, Marijuana Cannabis seed, and some vegetable seed.

Report of Analysis: The document issued by the laboratory, giving the final results of laboratory tests. Specific information required to be reported on the report is outlined in Section 1.2 and 1.3.

Seeds Act: The seed legislation passed by parliament which defines the standards which must be met to ensure the availability of pure, efficacious seed to Canadian consumers.

Seed Laboratory Audit and Accreditation Protocol (Seed LAAP): Defines the principle requirements for a seed testing laboratory to be accredited and maintain its accreditation.

Seeds Regulations: Refers to the "Regulations respecting the quality of seeds including seed potatoes, and the testing, inspection, and sale thereof.”

Weed Seeds Order: Refers to the list of plant species the seeds of which are deemed to be "weed seeds" pursuant to Section 6(2) of the Seeds Act. The Order is published as part of the Seeds Regulations.

Worksheet: Document used by the laboratory to record analytical results and other relevant information for the sample under test. The worksheet is normally an internal laboratory document, but may also serve as the report of analysis. It must contain details directly related to the analytical procedure used, such as purity working sample weights, germination method, etc., and must indicate who conducted the test or activity.

1.2 CONDITIONS FOR ISSUING REPORTS OF ANALYSIS

a. Results of analyses completed according to these *Canadian Methods and Procedures for Testing Seed* (M&P) must be reported on a report of analysis if the results are to be used for grading purposes. The assessment of any attribute for grading purposes reported on a report of analysis is to be determined from tests conducted on one submitted sample from one sampling operation of a lot.

b. The tests must have been conducted by the issuing laboratory or under sub-contract with the issuing laboratory (See Section 1.3.5).

c. Analytical results as required for grading according to the the Seeds Regulations and GradeTables.

d. If prohibited noxious weed seeds are found during the analysis of any sample or are observed in an unanalyzed portion, the laboratory, must notify Seed Section of the finding at **cfia.seed-semence.acia@canada.ca**. Include the name of the species identified, the crop kind in which

the prohibited noxious weed seed was found and the country of production of the seed lot. (See Section 3.9.8.b)

e. **Hemp or Marijuana (*Cannabis spp.*)** There is no longer a requirement to notify Seed Section when Cannabis spp. seed is found in a sample due to changes in Health Canada legislation.

f. The laboratory may permit the assignment of a pedigreed grade name on the report of analysis if:

(i) the report has a distinct and separate seed grading report section and it is clearly identified as a "Seed Grading Report." The Seed Grading Report section must contain the crop certificate number, the statement "accredited seed grader" followed by both the printed name and signature of the grader. The statement, "accredited grader number" followed by the grader's accreditation number, the grade and the date.

(ii) the assignment of a pedigreed grade name is made by an accredited seed grader, who is in the laboratory's employ.

In all other cases, the laboratory must not provide on the report of analysis a grade name or any statement concerning the standard met by the sample.

g. In the case of crop kinds listed in Schedule II to the *Seeds Regulations,* variety names must not be indicated on the report of analysis unless the seed is pedigreed seed and proof of pedigree/proof of certification accompanies the submitted sample. Examples of proof of pedigree/proof of certification include the following:

(i). For domestic pedigreed seed:
- Crop certificate number issued by CSGA, or
- Crop year and crop report sequence number.

(ii). For imported pedigreed seed:
- AOSCA certified seed:
  - Pedigreed Reference Number, or
- OECD certified seed:
  - OECD reference number (three-letter country code followed by a sequence number).

In the case of crop kinds not listed in Schedule II to the *Seeds Regulations*, a variety name may be used on the report of analysis if the seed is of that variety.

1.3 COMPLETION OF THE REPORT OF ANALYSIS

The report of analysis must be typewritten or machine printed and contain the following information:

1.3.1 General

a. Unique laboratory assigned number: an identification number which must be assigned to the sample and any related worksheets and the report;

b. Name of crop kind on which the reported analyses were conducted;

c. Name, address and a contact number (e.g. phone/facsimile/e-mail) of the laboratory issuing the Report;

d. CFIA seed laboratory accreditation statement and number as required by the current Seed LAAP;

e. A lot identifier, such as seed lot number, if known;

f. CSGA Crop Certificate Number, if known; (See Section 1.2.g)

g. Test method(s) used if different from those prescribed or where required to be reported, in these Methods and Procedures;

h. Signature of an Accredited Analyst; or the Head of the issuing laboratory or their assignee. It may be either a physical or a CFIA approved electronic signature;

i. Date test completed;

j. When an error has been found on a report of analysis after it has been sent out the amended report issued must state “Amended Report”.

k. Indicate on the report of analysis when a test was not undertaken or a component was not looked for by entering “-“ in the appropriate space.

1.3.2 Purity

a. Analysis of kinds not listed in the *Seeds Regulations*

- the approximate (estimated) number of seeds per gram must be reported in remarks as *“approximate number of seeds per gram – x.”* (See Section 2.3.5.a.iv)
- the total working sample weight (See Section 2.3.5.b.iii)

b. Where caryopses of the Poaceae are found as contaminants in a working sample (See Section 3.9.4)

- Unidentifiable caryopsis: the number present must be reported as "Poaceae sp." under Other Weed Seeds
- Free caryopses: which are not identifiable to species, but which can be identified as belonging to the genus *Elytrigia, Avena, Lolium*, or *Sorghum* must be classified and reported according to the appropriate noxious weed seed classification
- If no such evidence is present to support classification, report the genus name under Other Crop Seeds.

c. *Agropyron*, *Elymus, Elytrigia, Pascopyrum and/or Pseudoroegneria* species found as contaminants in non-wheatgrass samples (See Section 3.9.5)

- All contaminant seeds which are known to be slender wheatgrass (*Elymus trachycaulus*), and all seeds which cannot be distinguished by microscopic examination from slender wheatgrass, are to be reported as "slender wheatgrass".
- Any other seed of wheatgrass which is known not to be *Elymus repens* and cannot be identified to Genus level are reported as "Poaceae sp." under Other Crop Seeds.

d. Results obtained on tests to determine percentage by weight of components or impurities, (e.g. % pure seed, % inert, % ergot, etc.) (See Section 3.9.1.a and b)

- Must be given to one decimal place and must total 100.0%
- Results of less than 0.05% must be reported as trace or “TR”; 0.05% to 0.09% must be reported as 0.1%. If the percentage for a component is nil, this must be shown as "0.0" in the appropriate space.
- If a percentage for a component was not determined, this must be shown as "-" in the appropriate space.

Percentage Tests. When two or more tests are carried out on the same sample, report the weighted average of the compatible check tests. (See Section 3.9.6.a)

e. Results of tests for numbers per unit weight,

- The name and number of impurities expressed in terms of the unit weight as set out in the Grade Table (i.e. number per 25g, per 500g or per kg, depending on the Grade Table) must be shown. (See Section 3.9.2.a and b)

- If a fraction is obtained, do not round to a whole number (e.g. if 3 seeds are found in 50 grams analysed, report as 1.5 seeds per 25g, not as 2 per 25g). If the number of impurities

of a category is nil, this must be reported as "0". If the number of impurities of a category was not determined, this must be reported as "-". (See Section 3.9.2.b and c)

Tests for numbers of impurities per unit weight. When two or more tests are carried out on the same sample, or if a larger quantity than the minimum specified in Section 2.3 is analysed, then the total number of impurities in the total quantity analysed must be used in deriving the reported number of impurities per unit weight. (See Section 3.9.6.b)

f. **B*rassica* species and *Sinapis alba***, when found as impurities in Grade Tables VII, VIII, IX, X, XI, and XIII must be reported separately from and not included in the total other crop seeds. For all other Grade Tables, these species must be listed with and included in the total other crop seeds. (See Section 3.9.3.a)

g. **Cleavers or false cleavers (*Galium aparine* and *G. spurium*)**, when found in samples of the crops listed in Grade Table VII must be listed at the top of the "Secondary Noxious Weed Seeds" section, and include the number in the total secondary noxious. (See Section 3.9.3.c)

h. **Juncus tenuis (or *Juncus* spp. having seeds of a similar size)**, the presence but not the number of individual seeds must be reported under "Other Weeds" for Grade Table XII, XIV and XV and under "Remarks" for other Grade Tables. (See Section 3.9.3.f)

i. **Mayweed (*Anthemis cotula*) head**, when found in the kinds or species listed in Grade Table XVI report the number of heads or partial heads under "Secondary Noxious Weed Seeds" and include the number of heads in the total secondary noxious. Report the number of all loose seeds. (See Section 3.9.3.g)

j. **Sweet clover (*Melilotus albus* and *Melilotus officinalis*)**, for Grade Tables VIII, IX, X, XI, and XIII, sweet clover must be reported separately and not included in the total other crop seeds. For all other Grade Tables, sweet clover must be listed with and included in the total other crop seeds. (See Section 3.9.3.h)

k. **Tartarian buckwheat (*Fagopyrum tataricum*)**, when found in samples of the crops listed in Grade Tables I to III. List at the top of the "Other Crops" section, and include their number in the total other crops. (See Section 3.9.3.i)

l. **Wild oat (*Avena fatua* or *Avena sterilis*)**, when found in samples of the crops listed in Grade Tables I to III. List at the top of the "Secondary Noxious Weed Seeds" section, and include their number in the total secondary noxious. (See Section 3.9.3.j)

m. **Suspected varietal contaminants**, are to be noted under "Remarks", as in the following example. (See Section 3.9.7)

"Observed 5 yellow seeds in 500g analysed."

n. **Prohibited Noxious Weed Seeds observed in an Unanalyzed Portion**
Where a prohibited noxious weed seed is not found in the analyzed portion of a sample, but is observed in the unanalyzed portion, a statement must be made on the report of analysis indicating that the lot must not be graded due to the presence of a prohibited noxious weed seed(s). (See Section 3.9.8.b)

p. Procedure for Samples Too Small For Complete Analysis
When there is insufficient seed for a complete purity analysis, report the results on the report of analysis, and under "Remarks" include the statement: "Insufficient seed for complete analysis — do not grade" (See Section 3.9.8.d)

q. Reporting of species difficult to identify
When it is not possible to positively identify seed to the species level, report the genus name followed by 'sp', as in *Cuscuta* sp. If it is possible to distinguish it from seeds of another group within the genus, use 'sp.' followed in parentheses by 'cf.' and the name of the species which it most closely resembles, as in *Festuca* sp. (cf. *rubra*). Multiple species names may be used where there is greater uncertainty, as in *Festuca* sp. (cf. *rubra/brevipila*). Where common names are used, report as "Fescue (resembles red or hard)". When it is not possible to identify a seed to the genus level, report the family name followed by 'sp', as in Poaceae sp. (See Section 3.9.8.e)

1.3.3 Germination

a. **The germination result**, is to be reported as a percentage germination or germination plus hard seeds (See Section 4.10.7) calculated to the nearest whole number. (See Section 4.11.5.c) When a germination test was not undertaken, or a germination test component was not looked for, enter "-" in the appropriate space on the Report of Analysis.

b. **Pure Living Seed**, is to be reported as a percentage calculated to the nearest whole number. (See Section 4.11.4.b)

c. **When modified germination methods are used**, the method must be clearly indicated. (See Section 4.2.2.e)

d. **For mixtures**, of cereal seeds, Grade Table III, forage seeds, Grade Table XIII, lawn or turf mixtures, Grade Table XIV or ground cover mixtures, Grade Table XV, report the percentage germination of each kind separately. (See Section 4.4.2 b and c)

e. ***Pascopyrum smithii,* Western wheatgrass** (See Section 4.7.1.c)
The percent germination section of the report of analysis must show a dash (-). In "Remarks" the following statement must be made:

"Due to inherent dormancy in this species, the total of germination and dormant seed is to be used for grading purposes. Level of dormancy was determined by the tetrazolium test.

Germination	____ %
Dormant seed	____ %
Total germ + dormant	____ %

f. **Tetrazolium Testing Results**, are reported under "Remarks", or in a section designated for reporting of tetrazolium test results. (See Section 4.7.6)

g. **When Fungicides are used**, results of the untreated tests are reported, with the results of the treated tests reported in the 'remarks' section. (See Section 4.7.7)

h. **When a treatment for promoting germination of dormant seeds, is used as listed in the "Fresh or Dormant Seeds" column of Table 5** or an alternate method that is not listed for the kind under test in Table 5, its use must be indicated on the report of analysis. (See Section 4.8)

i. **When a test has been extended**, after the prescribed final count, the extension period must be reported. (See Section 4.9.3.c.iii)

j. **Frozen and Immature Cereals** when a sample is characterized by seedlings on the borderline of abnormality as a result of frost damage, or by spindly seedlings from immature seeds, or by these two conditions together, a statement to this effect should be made. (See Section 4.10.3)

k. Seedlings Infected With Fungi or Bacteria, The presence of disease should be noted but identification of the disease must only be made by someone with appropriate training. (See Section 4.10.4)

1.3.4 True Loose Smut

True Loose Smut result must be reported as percentage by number calculated to the nearest whole number. The infection must be reported using both scientific and common names as follows: "*Ustilago nuda* (True Loose Smut): [X]% embryos infected." (See Section 5.9)

1.3.5 Sub-contracted Results

A laboratory may enter into a sub-contract agreement with another laboratory accredited by CFIA.

a. The sub-contracted laboratory must conduct the test(s) by a method stated in the current Canadian Method and Procedures for Testing Seed.

b. The issuing laboratory must include on its report in the Remarks section, the test results provided, the name of the sub-contracted laboratory that conducted the testing, the sub-contracted laboratory's unique laboratory assigned number (report of analysis number) and the date the test was completed.

1.3.6 When the test method used is not within the laboratory's scope of accreditation by the CFIA.

The following must be stated on the report of analysis:

Where an AOSA or ISTA method is used, the name of the Rules (e.g. Tested in accordance with ISTA Rules) and the method.

a. Where the method used is not stated in the M&P, the AOSA Rules or the ISTA Rules the test method and a statement that there are no published methods for the test. (See Section 4.6.1)

b. Where an M&P test method is used and/or a kind or species is tested that is not in the laboratory's scope of accreditation by the CFIA, the name of the Rules, the test method and a statement that the test method or crop kind or species is not within the laboratory's CFIA scope of accreditation.

c. Where the method used is not stated in M&P, the weight of the working sample tested for purity and the approximate number of seeds per gram.

1.3.7 Retention of High-value seed

For high value seeds other than marijuana, upon the request of the customer, the seeds will be returned in full to the customer with the exception of the impurities found. On the "Report of Analysis" the following statement must be made in the "Remarks" section "Submitted sample returned to customer, no seed retained at lab from this sample to conduct a re-test." If customer desires a re-test a new sample must be submitted and Sections 1.3.7, 2.3.6 and 3.9.2 d apply.

2.0 SAMPLING

2.1 SAMPLING THE LOT

The procedures for sampling seed lots are based on the principles set out in the Canadian Food Inspection Agency's specific work instruction for sampling (SWI 132.1.1) issued by Seed Section:

http://www.inspection.gc.ca/plants/seeds/seed-inspection-procedures/swi-132-1-1/eng/1385748378305/1385748379805.

For the purpose of taking a sample for establishing the grade of pedigreed seed under the Seeds Regulations the procedures are described in the Canadian Seed Institute (CSI) Technical Manuals.

2.1.1 Size of Submitted Sample

"For crop kinds listed in Schedule I to the *Seeds Regulations*, the minimum size of the submitted sample will be that in Section 2.3.4 Table 1 of the M&P. For crop kinds not in Schedule I, the minimum size of submitted sample will be that in Section 2.3.5 of the M&P." See Section 2.3.6 for High-value seed where obtaining a submitted sample of prescribed weight is not possible because the size of the entire lot is smaller than the prescribed weight.

2.2 OBTAINING THE WORKING SAMPLE

The working sample is that part of the submitted sample on which the laboratory analyses are conducted (e.g. percent test, numbers per unit weight, germination). The working sample(s) must be taken from the submitted sample in such a manner that it will represent the entire sample as accurately as possible. A working sample(s) of not less than the minimum weight(s) required as set out in Section 2.3.4 Table 1 (e.g. Pure seed, 1st quantity, 2nd quantity, 3rd quantity and/or germination) is to be obtained. See Section 2.3.1 and 2.3.2.

A mechanical mixer or, preferably, a mechanical mixer - divider, should be used. If, however, a mechanical mixer is found to be unsuitable for the kind of seed being tested, then the hand mixing spoon method may be used.

The sampling procedures which must be used are described below:

2.2.1 Mixing and Dividing Procedures

Mechanical Divider Method

This method is suitable for most kinds of seeds. The apparatus divides a sample into two approximately equal parts. The submitted sample is mixed by passing it through the divider, recombining the two parts and passing the whole sample through a second time and similarly a third time. The sample then is reduced by passing the seed through repeatedly and removing one-half on each occasion. This process of successive halving is continued until a working sample of the required size is obtained.

Use of compressed air is highly recommended for cleaning mechanical dividers.

The dividers described below are examples of suitable equipment. Other devices may be used if it can be demonstrated that they provide an unbiased sub-sample.

a. Centrifugal Divider (Gamet type

This divider is suitable for all kinds of seed except oilseeds (such as rapeseed, canola, mustards, flax) and kinds susceptible to damage (such as peas, soybeans, etc) and the extremely chaffy types.This divider makes use of centrifugal force to mix and scatter seeds over the dividing surface. The centrifugal divider tends to give variable results when not carefully operated.

(i). Preparation of the apparatus:

Level the divider using the adjustable feet.
Check the divider and four containers for cleanliness. Note that seeds can be trapped under the spinner and become a source of contamination.

(ii). Sample mixing:
Place a container under each spout.
Feed the whole sample into the hopper; when filling the hopper, the seed must always be poured centrally.
After the sample has been poured into the hopper, the spinner is operated and the seed passes into the two containers. Turn off spinner
Full containers are replaced by empty containers. The contents of the two full containers are fed centrally into the hopper together, the seed being allowed to blend as it flows in. The spinner is operated.
The sample mixing procedure is repeated at least once more.

The sample is mixed a minimum of three times before sample reduction begins.

(iii). Sample reduction:
The two full containers are replaced by two empty containers. The contents of one full container are set aside and the contents of the other container are fed into the hopper. The spinner is operated.
The successive halving process is continued until the working sample(s) of not less than the minimum weight(s) required as set out in Section 2.3.4 Table 1 are obtained.
Ensure that the divider and containers are clean after each mixing operation.

b. Soil/Riffle Divider

This divider is suitable for most kinds of seed, including peas, beans, soybeans etc., provided the fall of the seed is not such that the seed will be damaged. For round-seeded kinds such as *Brassica*, the receiving pans should be covered to prevent the seeds from bouncing out.
This divider consists of a hopper with attached channels or ducts, a frame to hold the hopper, four collecting pans and a pouring pan. Ducts or channels lead from the hopper to the collecting pans, alternate ones leading to opposite sides. Riffle dividers are available in different sizes for different sizes of seed. The width and number of channels and spaces are important. The width of the channels must be at least two times the largest diameter of the seed or any possible contaminants being mixed.

This apparatus, similar to the centrifugal divider, divides the sample into approximately equal parts.

(i). Preparation of the apparatus:
Place the riffle divider on a firm, level clean surface. Ensure the divider is level.
Ensure that the divider and the 4 sample collection containers are clean. Check all channels, joints and seams of the divider and collection containers to ensure there are no seeds or other plant matter present before each use.
Two clean empty collection containers are placed under the channels to receive the mixed seed.

(ii). Sample mixing:
Pour the whole sample into the divider by running the seed backwards and forwards along the edge of the divider so that all the channels and spaces of the divider receive an equal amount of seed.
The two full containers are replaced with two clean empty collection containers.
The contents of one full collection container is poured into the divider by holding the long edge of the pan against the long edge of the riffle hopper and then rotating the bottom up so that the seeds pour across all channels at the same time; followed by the other full container using the same procedure.
This process of mixing the entire submitted sample must be repeated at least 2 more times before successive halving begins.

(iii). Sample reduction:
The contents of one full collection container are set aside. Empty collection containers are placed under each channel and the contents of the other container is poured into the hopper by holding the long edge of the pan against the long edge of the riffle hopper and then rotating the bottom up so that the seeds pour across all channels at the same time.
The successive halving process is continued until the working sample(s) of not less than the minimum weight(s) required as set out in Section 2.3.4 Table 1 are obtained.

Ensure that the divider and collection containers are clean after each mixing operation. Check all channels of the divider, the joints and seams.

2.2.2 Hand Mixing Spoon Method

This method should only be used for samples of a single small-seeded species that are smaller than *Triticum* spp., very chaffy species or uncleaned seed where it is demonstrated that one of the mechanical dividers will not take a representative working sample(s). The sample is poured uniformly over a tray with a side to side swinging motion. The receiving pan should be kept level. This mixing procedure is repeated a minimum of 3 times.
A tray, a spatula and a spoon with a straight edge are required. After the preliminary mixing, pour the seed evenly over the tray with a side-to-side swing, alternately in one direction and at right angles to it. The depth of the seed in the pan must not exceed the height of the vertical sides of the spoon. Do not shake the tray thereafter. With the spoon in one hand, the spatula in the other, and using both, remove small portions of seed from not less than five random places on the tray. Sufficient portions of seed are taken to constitute a working sample(s) of the required size as set out in Section 2.3.4 Table 1.

2.3 WORKING SAMPLE WEIGHTS

Working samples of the required size are drawn from the submitted sample using the procedures outlined in Section 2.2. Weights of working samples are determined from Section 2.3.4 Table 1.

2.3.1 Weights for analysis for numbers per unit weight

When the analysis is for numbers of impurities (weed seeds of the different classes, sweet clover, other crop seeds, ergot, etc.) per unit weight, the total working sample weight is the total of the quantities given in columns 3, 4 and 5 of Section 2.3.4 Table 1. At the time of sub-sampling from the submitted sample, the working sample is usually divided into the different weight portions given in the Table so that a sequential analysis can be followed, as described in Section 3.6.2. The weights of the portions must be as close as possible to the weights given in the columns of Table 1, with a deviation of no more than + 2% of the specified weight.

2.3.2 Weights for analysis for percentage by weight

When the analysis is for percentage by weight of one or more components or impurities (pure seed, other crop seeds, weed seeds, sweet clover, inert matter, etc.), the determination is made on the weight specified in column 6 of Section 2.3.4 Table 1. This quantity may be extracted from one of the working portions to be used for the determination of numbers of impurities per unit weight. For percentage by weight determinations at a minimum, the weight specified in column 6 must be analysed with a deviation of no more than + 5% of the specified weight.

If it is necessary to perform a check test because the percentage determined for one or more factors is "on-the-line", the quantity to be used is equivalent to that given in column 6 of Table 1. This quantity must be drawn in the same manner as for the first test.

2.3.3 Weights for analysis of mixtures

a. Mixture characteristics. For samples of mixtures, the normal mixing and dividing procedures in Section 2.2 are used.

b. Seed Size. For purposes of this section, seed of the crop kinds listed in Grade Tables VIII to XII or kinds or species of similar size must be considered as belonging to the following size categories:

Type of mixture	**Kinds considered large-seeded**	**Kinds considered small-seeded**
Forage Mixtures (Grade Table XIII) Mixtures that are comprised of kinds or species listed in the Grade Tables	All species listed in Grade Table VIII; bromegrass; meadow and tall fescue; the wild-ryes; the ryegrasses; the wheatgrasses, except crested.	All species listed in Grade Tables IX to XII which are not considered large-seeded.
Forage Mixtures (Grade Table XIII as defined in the Seed Regulations 6 (2)) (labelled for land reclamation, soil conservation, green cover, wildlife grazing or habitat, wetland restoration and similar purposes)	See Section 2.3.5 and 3.4.1 for mixtures that contain kinds or species: • not listed in the Grade Tables; or • not listed in the Grade Tables and listed in the Grade Tables	
Lawn or turf (Grade Table XIV)	All species permitted under Grade Table XIV, except *Poa* spp., *Agrostis* spp., white clover and Timothy.	*Poa* spp., *Agrostis* spp., white clover and Timothy.
Lawn or turf (Grade Table XIV)	See Section 2.3.5 and 3.4.1 for mixtures that contain kinds or species: • not listed in the Grade Tables; or • not listed in the Grade Tables and listed in the Grade Tables	
Ground Cover (Grade Table XV) Mixtures that are comprised of kinds or species listed in the Grade Tables	All species equal to or larger in size than those listed as large-seeded for forage mixtures.	All species which are not considered large-seeded.
Ground Cover (Grade Table XV) (includes mixtures containing wildflowers and similar products intended for landscape gardening use)	See Section 2.3.5 and 3.4.1 for mixtures that contain kinds or species: • not listed in the Grade Tables; or • not listed in the Grade Tables and listed in the Grade Tables	

2.3.4 Table 1. Weights of Working Samples for Purity Analysis

a. For kinds not listed in Seeds Regulations Schedule I see Section 2.3.5

b. For the grading of pedigreed seed of Grade Tables I to VI, larger quantities than indicated in this table may be required. Refer to the Canadian Food Inspection Agency specific work instruction for seed sampling (SWI 132.1.1) for quantities to be analyzed and method of analysis.

Key for Column Headings of Table 1 (see also section 3.6).
Add weights in columns 3, 4 and 5 together for total weight to be analysed for impurities by number.

Column 1. Grade Table numbers.

Column 2. Kind of seed.

Column 3. **First quantity.** Analyse this quantity for all impurities set forth as numbers per kg, 500g or 25g in the Grade Tables. Where the analysis exceeds the line for rejection (See Section 3.6.3.b), the analysis may be terminated.

Column 4. **Second quantity.** If not rejected:

a. For all Foundation and Registered seed grades analyse this quantity for all impurities.

b. For Certified seed grades Grade Tables I to VII analyze this quantity for all impurities.

c. In all other cases (except Grade Tables XIV and XV), analyze this quantity for all noxious weed seeds and for any other category of impurity which is on the line. When the analysis exceeds the line for rejection (See Section 3.6.3.b), the analysis may be terminated.

Note: Column 4 does not apply to Grade Table XIV or XV.

Column 5. **Third quantity.** If not rejected, analyze this quantity for prohibited and primary noxious weed seeds and any other category of impurity which is on the line.

Column 6. **Quantity for % purity.** Analyse this quantity for the percentage of pure seed, weed seeds, other crop seeds, ergot and/or sclerotia when required (See Sec. 3.5). Perform a check test on an equivalent quantity if "on the line". Where the analysis exceeds the line for rejection (See Section 3.5.3.b or 3.5.3.c), the analysis may be terminated.

TABLE 1:

1	2	3	4	5	6	7
Grade Table	Kind of seed	First quantity (g)	Second quantity (g)	Third quantity (g)	Quantity for % purity (g)	Additional information
I	*Triticum aestivum* Wheat, common	250	250	500	-	
	Triticum turgidum subsp. *durum* Wheat, durum	250	250	500	-	
II	*Avena nuda* Oat, hulless	250	250	500	-	
	A*vena sativa* Oat	250	250	500	-	
	Fagopyrum esculentum Buckwheat, common	250	250	500	-	
	Fagopyrum tataricum Buckwheat, tartarian	250	250	500	-	

1	2	3	4	5	6	7
Grade Table	Kind of seed	First quantity (g)	Second quantity (g)	Third quantity (g)	Quantity for % purity (g)	Additional information
II	*Hordeum vulgare* subsp. v*ulgare* Barley (six-row, two-row, hulless)	250	250	500	-	
	Lens culinaris Lentil	125	125	250	-	
	Lupinus spp Lupine, lupin (grain and forage)	125	125	250	-	
	Secale cereale Rye	250	250	500	-	
	Triticum aestivum subsp.spelta Spelt	250	250	500	-	
	Triticum turgidum subsp. *dicoccum* Emmer	250	250	500	-	
	Vigna radiata var. *radiata* Bean, mung	125	125	250	-	
	x Triticosecale spp. Triticale	250	250	500	-	
II.1	*Onobrychis viciifolia* Sainfoin	125	125	250	-	
	Vicia pannonica Vetch, Hungarian	125	125	250	-	See Section 3.8.8
	Vicia sativa subsp. *sativa* Vetch, common	125	125	250	-	See Section 3.8.8
	Vicia villosa subsp. *villosa* Vetch, hairy	125	125	250	-	See Section 3.8.8
III	Cereal mixtures	250	250	500	100	
IV	*Cannabis sativa* subsp. *sativa* Hemp	125	125	250	-	
	Linum usitatissimum oilseed flax or fibre	25	25	50	-	
	Phalaris canariensis Canarygrass	12.5	12.5	25	-	
	Sorghum bicolor Sorghum	25	25	50	-	
	Sorghum xdrummondii Sorghum Sudan grass hybrids	25	25	50	-	
	Sorghum xdrummondii Sudangrass	12.5	12.5	25	-	

1	2	3	4	5	6	7
Grade Table	Kind of seed	First quantity (g)	Second quantity (g)	Third quantity (g)	Quantity for % purity (g)	Additional information
V	*Cicer arietinum* Chickpea	125	125	250	-	
	Carthamus tinctorius Safflower	250	250	500	-	
	Glycine max Soybean	250	250	500	-	
	Helianthus annuus Sunflower (open-pollinated)	250	250	500	-	
	Phaseolus vulgaris Bean, field	250	250	500	-	
	Pisum sativum Pea, field	250	250	500	-	
	Vicia faba Bean, broad	250	250	500	-	
	Vicia faba Bean, faba	250	250	500	-	
	Vicia faba Bean, horse	250	250	500	-	
	Vicia faba Bean, tick	250	250	500	-	
	Vigna unguiculata subsp. *unguiculata* Cowpea	125	125	250	-	
	Zea mays subsp. *mays* Corn (open-pollinated, synthetic)	250	250	500	-	
VI	*Helianthus annuus* Sunflower, hybrid	250	250	500	-	
	Zea mays subsp. mays Corn, hybrid	250	250	500	-	
VII	*Brassica juncea* Mustard, brown	12.5	12.5	25	-	See Section 3.8.5
	Brassica juncea Mustard, Indian	12.5	12.5	25	-	See Section 3.8.5
	Brassica juncea Mustard, oriental	12.5	12.5	25	-	See Section 3.8.5
	Brassica juncea Rapeseed, oilseed rape, canola – *Brassica juncea* type	12.5	12.5	25	-	See Section 3.8.5

1	2	3	4	5	6	7
Grade Table	Kind of seed	First quantity (g)	Second quantity (g)	Third quantity (g)	Quantity for % purity (g)	Additional information
VII	*Brassica napus* var. *napus* Rapeseed, oilseed rape, canola - Argentine type	12.5	12.5	25	-	See Section 3.8.5
	Brassica nigra Mustard, black	12.5	12.5	25	-	See Section 3.8.5
	Brassica rapa subsp. *campestris* Rapeseed, oilseed rape, canola - Polish type	12.5	12.5	25	-	See Section 3.8.5
	Sinapis alba Mustard white	25	25	50	-	See Section 3.8.5
	Raphanus sativus var. *oleiformis* Radish, oilseed or forage	75	75	150	-	
VIII	*Anthyllis vulneraria* Vetch, kidney	12.5	12.5	25	2.5	
	Astragalus cicer Milk-vetch, cicer	12.5	12.5	25	2.5	
	Echinochloa esculenta Millet, Japanese	12.5	12.5	25	2.5	
	Echinochloa frumentacea Millet, Japanese	12.5	12.5	25	2.5	
	Lespedeza cuneata Lespedeza, sericea or Chinese	12.5	12.5	25	2.5	
	Kummerowia stipulacea Lespedeza, Korean	12.5	12.5	25	2.5	
	Kummerowia striata Lespedeza, common or Kobe	12.5	12.5	25	2.5	
	Medicago sativa Alfalfa	12.5	12.5	25	2.5	
	Melilotus albus Clover, sweet - white blossom	12.5	12.5	25	2.5	
	Melilotus officinalis Clover, sweet - yellow blossom	12.5	12.5	25	2.5	
	Panicum miliaceum subsp. *miliaceum* Millet, proso	12.5	12.5	25	2.5	
	Pennisetum glaucum Millet, pearl	12.5	12.5	25	2.5	
	Securigera varia Vetch, crown	12.5	12.5	25	2.5	
	Setaria italica subsp. *italica* Millet, foxtail or Italian	12.5	12.5	25	2.5	

1	2	3	4	5	6	7
Grade Table	Kind of seed	First quantity (g)	Second quantity (g)	Third quantity (g)	Quantity for % purity (g)	Additional information
VIII	*Trifolium incarnatum* Clover, crimson	12.5	12.5	25	2.5	
	Trifolium pratense Clover, red	12.5	12.5	25	2.5	
	Trifolium subterraneum Clover, subterranean	12.5	12.5	25	2.5	
IX	*Medicago lupulina* Medick, black	6.25	6.25	12.5	1	
	Phleum bertolonii Timothy - dwarf	6.25	6.25	12.5	1	
	Phleum pratense Timothy - common	6.25	6.25	12.5	1	
	Trifolium aureum Clover, hop or yellow	6.25	6.25	12.5	1	
	Trifolium campestre Clover, hop, large	6.25	6.25	12.5	1	
	Trifolium dubium Clover, hop, small (suckling)	6.25	6.25	12.5	1	
	Trifolium fragiferum Clover, strawberry	6.25	6.25	12.5	1	
	Trifolium hybridum Clover, alsike	6.25	6.25	12.5	1	
	Trifolium repens Clover, white	6.25	6.25	12.5	1	
	Trifolium resupinatum Clover, Persian	6.25	6.25	12.5	1	
X	*Lotus corniculatus* Trefoil, bird's-foot	6.25	6.25	12.5	1	
XI	*Agropyron cristatum* Wheatgrass, crested	6.25	6.25	12.5	1	See Section 3.8.2.a
	Agropyron desertorum Wheatgrass, crested	6.25	6.25	12.5	1	See Section 3.8.2.a
	Agropyron fragile subsp. *sibiricum* Wheatgrass, Siberian	6.25	6.25	12.5	1	See Section 3.8.2.a
	Alopecurus arundinaceus Foxtail, creeping	3.13	3.13	6.25	0.5	
	Alopecurus pratensis Foxtail, meadow	3.13	3.13	6.25	0.5	
	Arrhenatherum elatius Oatgrass, tall	6.25	6.25	12.5	1	

1	2	3	4	5	6	7
Grade Table	Kind of seed	First quantity (g)	Second quantity (g)	Third quantity (g)	Quantity for % purity (g)	Additional information
XI	*Bromus carinatus* Bromegrass, sweet	12.5	12.5	25	2	
	Bromus inermis Bromegrass, smooth	12.5	12.5	25	2	
	Bromus riparius Bromegrass, meadow	12.5	12.5	25	2	
	Dactylis glomerata Orchardgrass	6.25	6.25	12.5	3	See Section 3.7.4
	Elymus dahuricus Wildrye, Dahurian	12.5	12.5	25	2	See Section 3.8.2.a
	Elymus lanceolatus Wheatgrass, northern	12.5	12.5	25	2	See Section 3.8.2.a and 3.8.2.c
	Elymus lanceolatus subsp. *lanceolatus* Wheatgrass, streambank	12.5	12.5	25	2	See Section 3.8.2.a and 3.8.2.c
	Elymus trachycaulus Wheatgrass, slender	12.5	12.5	25	2	See Section 3.8.2.a and 3.8.2.c
	Elytrigia elongata Wheatgrass, tall	12.5	12.5	25	2	See Section 3.8.2.a
	Elytrigia intermedia subsp. *intermedia* Wheatgrass, intermediate	12.5	12.5	25	2	See Section 3.8.2.a
	Elytrigia intermedia subsp. *intermedia* Wheatgrass, pubescent	12.5	12.5	25	2	See Section 3.8.2.a
	Festuca arundinacea Fescue, tall	12.5	12.5	25	2	See Section 3.8.6
	Festuca brevipila Fescue, hard	6.25	6.25	12.5	1	
	Festuca filiformis Fescue, fine-leaved	6.25	6.25	12.5	1	
	Festuca heterophylla Fescue, various leaved	6.25	6.25	12.5	1	
	Festuca ovina Fescue, sheep	6.25	6.25	12.5	1	
	Festuca pratensis Fescue, meadow	12.5	12.5	25	2	See Section 3.8.6
	Festuca rubra subsp. *fallax* Fescue, Chewing's	6.25	6.25	12.5	1	
	Festuca rubra subsp. *rubra* Fescue, red and creeping red	6.25	6.25	12.5	1	

1	2	3	4	5	6	7
Grade Table	Kind of seed	First quantity (g)	Second quantity (g)	Third quantity (g)	Quantity for % purity (g)	Additional information
XI	*Leymus angustus* Wildrye, Altai	12.5	12.5	25	2	See Section 3.8.2.a
	Lolium multiflorum Ryegrass, annual	12.5	12.5	25	2	See Section 3.8.7
	Lolium perenne Ryegrass, perennial	12.5	12.5	25	2	See Section 3.8.7
	Lolium x *hybridum* Ryegrass, intermediate	12.5	12.5	25	2	See Section 3.8.7
	Pascopyrum smithii Wheatgrass, western	12.5	12.5	25	2	See Section 3.8.2.a
	Phalaris arundinacea Canarygrass, reed	6.25	6.25	12.5	1	
	Psathyrostachys juncea Wildrye, Russian	12.5	12.5	25	2	See Section 3.8.2.a
	Pseudoroegneria spicata Wheatgrass, beardless	12.5	12.5	25	2	See Section 3.8.2.a
XII	*Agrostis canina* Bentgrass, velvet	1.56	1.56	3.13	0.5	See Section 3.7.3 and 3.8.3
	Agrostis capillaris Bentgrass, colonial (browntop)	1.56	1.56	3.13	0.5	See Section 3.7.3 and 3.8.3
	Agrostis gigantea Redtop	1.56	1.56	3.13	0.5	See Section 3.7.3 and 3.8.3
	Agrostis stolonifera Bentgrass, creeping	1.56	1.56	3.13	0.5	See Section 3.7.3 and 3.8.3
	Cynosurus cristatus Crested dogtail	6.25	6.25	12.5	1	
	Poa annua Bluegrass, annual	3.13	3.13	6.25	1	See Section 3.7.2 and 3.8.4
	Poa compressa Bluegrass, Canada	3.13	3.13	6.25	1	See Section 3.7.2 and 3.8.4
	Poa nemoralis Bluegrass, wood	3.13	3.13	6.25	1	See Section 3.7.2 and 3.8.4
	Poa palustris Bluegrass, fowl	3.13	3.13	6.25	1	See Section 3.7.2 and 3.8.4
	Poa pratensis Bluegrass, Kentucky	3.13	3.13	6.25	1	See Section 3.7.2 and 3.8.4
	Poa supina Bluegrass, supina	3.13	3.13	6.25	1	See Section 3.7.2 and 3.8.4

1	2	3	4	5	6	7
Grade Table	Kind of seed	First quantity (g)	Second quantity (g)	Third quantity (g)	Quantity for % purity (g)	Additional information
XII	*Poa trivialis* Bluegrass, rough	3.13	3.13	6.25	1	See Section 3.7.2 and 3.8.4
	Puccinellia distans Alkaligrass, weeping	3.13	3.13	6.25	1	
XIII	Mixtures of forage seeds (kinds listed in Grade Tables VIII to XII)					
	Mixtures of large seeds	12.5	12.5	25	2.5	
	Mixtures of small seeds	6.25	6.25	12.5	2	
	Mixtures of large and small seeds					
	Mixtures of seeds composed of or, containing kinds not listed in the Grade Tables	-	-	-	-	See Section 2.3.5
XIV	Lawn or turf mixtures					
	Mixtures of large seeds	6.25	-	6.25	1	
	Mixtures of small seeds	3.13	-	3.13	1	
	Mixtures of large and small seeds	-	-	-	-	See Section 3.7.2.c and 3.7.5
	Mixtures of seeds composed of or, containing kinds not listed in the Grade Tables	-	-	-	-	See Section 2.3.5 and 3.7.5
XV	Ground cover mixtures					
	Mixtures of large seeds	12.5	-	12.5	2	
	Mixtures of small seeds	6.25	-	6.25	1	
	Mixtures of large and small seeds	-	-	-	-	
	Mixtures of seeds composed of or, containing kinds not listed in the Grade Tables.	-	-	-	-	See Section 2.3.5
XVI	*Beta vulgaris* subsp. *vulgaris* Beet	125	125	-	-	
	Beta vulgaris subsp. *vulgaris* Beet, sugar	250	250	-	-	
	Beta vulgaris subsp. *vulgaris* Mangel	125	125	-	-	
	Beta vulgaris subsp. *vulgaris* Swiss chard	125	125	-	-	
XVII	*Citrullus lanatus* var. *citroides* Citron	12.5	12.5	-	-	
	Citrullus lanatus var. *lanatus* Watermelon	12.5	12.5	-	-	

1	2	3	4	5	6	7
Grade Table	Kind of seed	First quantity (g)	Second quantity (g)	Third quantity (g)	Quantity for % purity (g)	Additional information
XVII	*Cucumis melo* subsp. *melo* Cantaloupe, melon	12.5	12.5	-	-	
	Cucumis anguria Gherkin	12.5	12.5	-	-	
	Cucumis sativus Cucumber	12.5	12.5	-	-	
	Cucurbita spp. Pumpkin, squash (summer or winter)	12.5	12.5	-	-	
XVIII	*Carthamus tinctorius* Safflower	250	250	-	-	
	Cicer arietinum Chickpea	125	125	-	-	
	Glycine max Soybean	250	250	-	-	
	Helianthus annuus Sunflower	250	250	-	-	
	Phaseolus coccineus Bean, runner	250	250	-	-	
	Phaseolus lunatus Bean, lima	250	250	-	-	
	Phaseolus vulgaris Bean, garden	250	250	-	-	
	Pisum sativum Pea, garden	250	250	-	-	
	Vicia faba Bean, broad	250	250	-	-	
	Zea mays subsp. *mays* Corn, pop or sweet	250	250	-	-	
XIX	*Brassica juncea* Mustard greens	6.25	6.25	-	-	See Section 3.8.5
	Brassica oleracea var. *botrytis* Cauliflower	3.13	3.13	-	-	See Section 3.8.5
	Brassica oleracea var. *capitata* Cabbage	3.13	3.13	-	-	See Section 3.8.5
	Brassica oleracea var. *gemmifera* Brussels sprouts	3.13	3.13	-	-	See Section 3.8.5
	Brassica oleracea var. *gongylodes* Kohlrabi	3.13	3.13	-	-	See Section 3.8.5

1	2	3	4	5	6	7
Grade Table	Kind of seed	First quantity (g)	Second quantity (g)	Third quantity (g)	Quantity for % purity (g)	Additional information
XIX	*Brassica oleracea* var. *italica* Broccoli	3.13	3.13	-	-	See Section 3.8.5
	Brassica oleracea var. *sabellica* Kale (garden / ornamental)	3.13	3.13	-	-	See Section 3.8.5
	Brassica oleracea var. *viridis* Collards (forage)	12.5	12.5	25	-	See Section 3.8.5
	Brassica oleracea var. *viridis* Kale (garden / ornamental)	3.13	3.13	-	-	See Section 3.8.5
	Brassica napus var. *napobrassica* Rutabaga (swede)	6.25	6.25	-	-	See Section 3.8.5
	Brassica napus var. *napus* Rape, forage	6.25	6.25	-	-	See Section 3.8.5
	Brassica rapa subsp. *campestris* Rape, forage	6.25	6.25	-	-	See Section 3.8.5
	Brassica rapa subsp. *chinensis* Cabbage, Chinese	6.25	6.25	-	-	See Section 3.8.5
	Brassica rapa subsp. *pekinensis* Cabbage, Chinese	6.25	6.25	-	-	See Section 3.8.5
	Brassica rapa var. *perviridis* Spinach mustard	3.13	3.13	-	-	See Section 3.8.5
	Brassica rapa subsp. *rapa* Turnip	6.25	6.25	-	-	See Section 3.8.5
	Raphanus sativus Radish	12.5	12.5	-	-	
XX	*Abelmoschus esculentus* Okra	12.5	12.5	-	-	
	Allium cepa Onion	12.5	12.5	-	-	
	Allium porrum Leek	6.25	6.25	-	-	
	Allium schoenoprasum Chives	6.25	6.25	-	-	
	Anethum graveolens Dill	6.25	6.25	-	-	
	Anthriscus cerefolium Chervil	6.25	6.25	-	-	
XX	*Apium graveolens* var. *dulce* Celery	3.13	3.13	-	-	

1	2	3	4	5	6	7
Grade Table	Kind of seed	First quantity (g)	Second quantity (g)	Third quantity (g)	Quantity for % purity (g)	Additional information
	Apium graveolens var. *rapaceum* Celeriac	3.13	3.13	-	-	
	Asparagus officinalis Asparagus	12.5	12.5	-	-	
	Campanula rapunculus Rampion	1.56	1.56	-	-	
	Capsicum annuum Pepper	6.25	6.25	-	-	
	Cichorium endivia Endive	6.25	6.25	-	-	
	Cichorium intybus Chicory, cultivated	6.25	6.25	-	-	
	Cynara cardunculus Artichoke	12.5	12.5	-	-	
	Cynara cardunculus Cardoon	12.5	12.5	-	-	
	Daucus carota subsp. *sativus* Carrot, cultivated	12.5	12.5	-	-	
	Lactuca sativa var. angustana Celtuce, Celtus	6.25	6.25	-	-	
	Lactuca sativa var. *capitata* Lettuce – head	6.25	6.25	-	-	
	Lactuca sativa var. crispa Lettuce - leaf	6.25	6.25	-	-	
	Lactuca sativa var. *longifolia* Lettuce - cos (romaine)	6.25	6.25	-	-	
	Lepidium sativum Cress, garden	6.25	6.25	-	-	
	Lycopersicon esculentum Tomato	6.25	6.25	-	-	
	Nasturtium officinale Cress, water	1.56	1.56	-	-	
	Nicotiana tabacum Tobacco, flue-cured and burley types	1.56	1.56	-	-	
	Pastinaca sativa subsp. s*ativa* Parsnip	12.5	12.5	-	-	
	Petroselinum crispum Parsley	6.25	6.25	-	-	

1	2	3	4	5	6	7
Grade Table	Kind of seed	First quantity (g)	Second quantity (g)	Third quantity (g)	Quantity for % purity (g)	Additional information
XX	*Rheum* x *hybridum* Rhubarb	6.25	6.25	-	-	
	Rumex acetosa Sorrel, cultivated	3.13	3.13	-	-	
	Salvia officinalis Sage	6.25	6.25	-	-	
	Satureja hortensis Savory	3.13	3.13	-	-	
	Satureja montana Savory	3.13	3.13	-	-	
	Solanum melongena Eggplant	6.25	6.25	-	-	
	Spinacia oleracea Spinach	12.5	12.5	-	-	
	Taraxacum officinale Dandelion, cultivated	3.13	3.13	-	-	
	Tetragonia tetragonoides Spinach, New Zealand	12.5	12.5	-	-	
	Thymus vulgaris Thyme	1.56	1.56	-	-	
	Tragopogon porrifolius Salsify	12.5	12.5	-	-	
	Valerianella locusta Cornsalad	6.25	6.25	-	-	

2.3.5 Weights for Analysis of kinds not listed in the Seeds Regulations

The purity analysis must be made on a working sample taken from the submitted sample in accordance with Section 2.2.

a. Determination of number of seeds per gram
Use one of the following to determine the number of seeds per gram:

(i). use the approximations for numbers of seeds per gram given in Table 2A of the current AOSA Rules for Testing Seeds. Where a range is given, use the mid-point (e.g. if the range is 1500-2000, use 1750).

(ii). if the kind is not listed in the AOSA Rules, calculate the number of seeds per gram from the purity working sample weights given in the current ISTA Rules for Seed Testing, Table 2A, Column - *Working sample for purity analysis*. The ISTA purity working samples contain approximately 2500 seeds, therefore 2500 divided by Table 2A, Column - *Working sample for purity analysis*, weight will give the approximate number of seeds per gram.

(iii). if the kind is not listed in the AOSA Rules or the ISTA Rules, estimate the number of seeds per gram by weighing 500 pure seeds taken at random from the sample. The number of seeds per gram will equal 500 divided by the weight of the 500 seeds.

Example:
Weight of 500 pure seeds = 1.0103 grams

$$\text{Number of seeds per gram} \frac{500}{1.0103\ \text{g}} = 494.9 \blacktriangleright 495 \text{ seeds per gram}$$

(iv). the approximate (estimated) number of seeds per gram must be reported in remarks on the report of analysis as *"approximate number of seeds per gram – x."*

b. Working sample size.

1	2	3
No. of seeds per gram	**Count of Noxious Working Sample Size**	**Full Species/Percentage Test Working Sample Size**
Less than 25	1000 grams	100 grams
25 or more	25000 seeds	2500 seeds

Where the kind or species is:

(i). listed in the current AOSA Rules for Testing Seeds the weights in Table 2A may be used for testing and reporting. (See note**)

(ii). not listed in the AOSA Rules but listed in the current ISTA Rules for Seed Testing the weights in Table 2A of the ISTA Rules may be used for testing. Where the ISTA Rules does not state a quantity for "other seeds by number", 10x the weight stated for "purity analysis" must be the working sample size for the count of other species. (See note**)

**** Note: Where the working sample size stated in AOSA or ISTA Rules is less than the minimum requirement for working sample size stated in the table above for less than 25 seeds per gram the working sample size, at a minimum, stated in the above table must be analysed.**

(iii). When the species is not in the AOSA Rules or the ISTA Rules, the weights of a working sample for 2500 and 25000 seeds must be calculated based on the determination of the number of seeds per gram.

Example working sample size calculation for those kinds not listed in Schedule I:
(Number of seeds per gram was determined to be 495 seeds)

Working sample size for:
Count of noxious species working sample size: 25000 / 495 = 51 grams
Full species/Percentage Test working sample size: 2500 / 495 = 5.1 grams

(iv). the total working sample weight must be reported on the report of analysis

c. Mixtures of seeds containing:

- kinds not listed in the Grade Tables
- kinds not listed in the Grade Tables and listed in the Grade Tables.

(i). Obtaining the working sample

The purity analysis must be made on a working sample taken from the submitted sample in accordance with Section 2.2. The spoon method may be used if the spoon method is allowed for at least one of the components in the mixture.

(ii). Determination of the number of seeds per gram

The estimation and reporting of the number of seeds per gram must be as stated in Section 2.3.5.a.

(iii). Determination of the working sample size for 25000 seeds

The procedures for the determination of the working sample size are as stated in the

1. the current AOSA Rules for Testing Seeds 2.3.b.(4), if all of the species are listed in the AOSA Rules. See Section 2.3.5.b ** Note.
2. the current ISTA Rules Chapter 18, if all or some of the species are not listed in the AOSA Rules. See Section 2.3.5.b ** Note.

2.3.6 Small seed lots of High-value Seed

In cases of High-value seed as described in Section 2.1.1, a reduced test can be performed. In a reduced test, less than the prescribed working sample weight is examined for all other seeds.

3.0 PURITY ANALYSIS

3.1 INTRODUCTION

In most seed laboratories throughout the world, purity analysis means the determination of the percentage by weight of pure seed, other seeds and inert matter. In Canada, however, such determinations do not fully meet the requirements of the grading system as prescribed by the Seeds Act and Regulations. The principal criteria of purity employed in the Canadian grading system are based on the number of weed seeds, other crop seeds and other impurities per unit weight, although in some cases percentages by weight may also be grading factors. The Seeds Regulations and the Grade Tables indicate the criteria of purity used in grading the various kinds of crop seeds to which the Regulations apply.

3.2 DEFINITIONS

3.2.1 Seed

A seed, in laboratory practice, is defined as "a structure which contains at least one ripened ovule with or without accessory parts".

In many crop plants, the structure commonly regarded as the seed is botanically a fruit. Thus, in addition to true seeds, the foregoing definition includes florets and caryopses in the Poaceae, achenes, cypselas, schizocarps, mericarps, nutlets, one- and two-seeded pods of small-seeded legumes, seed balls, or portions thereof, in *Beta* spp., fruits with enclosing calyx as in *Tetragonia tetragonoides.* Bulblets, such as those of *Poa bulbosa,* are also considered seeds, although they do not contain an ovule, because they can grow into a plant if sown. It also includes coated seed.

Very often structures which do not strictly comply with the above definition are included in the pure seed because the analyst cannot tell whether or not the ripened ovule is present.

Single and multiple florets are defined as follows (see Figure 2). The length of an awn must be disregarded when determining the length of a fertile floret or an attached structure.

(i). Single floret: A fertile floret with or without attached single fertile or sterile floret, provided that the attached floret does not extend to the tip of the fertile floret, excluding the awn (structures 1-4).

(ii). Multiple floret: Any of the following structures:
- A fertile floret with two or more attached sterile and/or fertile florets of any length (structures 5-7);
- A fertile floret with an attached sterile or fertile floret that extends to or beyond the tip of the fertile floret (structures 8-12);
- A fertile floret with basally attached glume or glumes, or basally attached sterile floret of any length (structures 13-15).

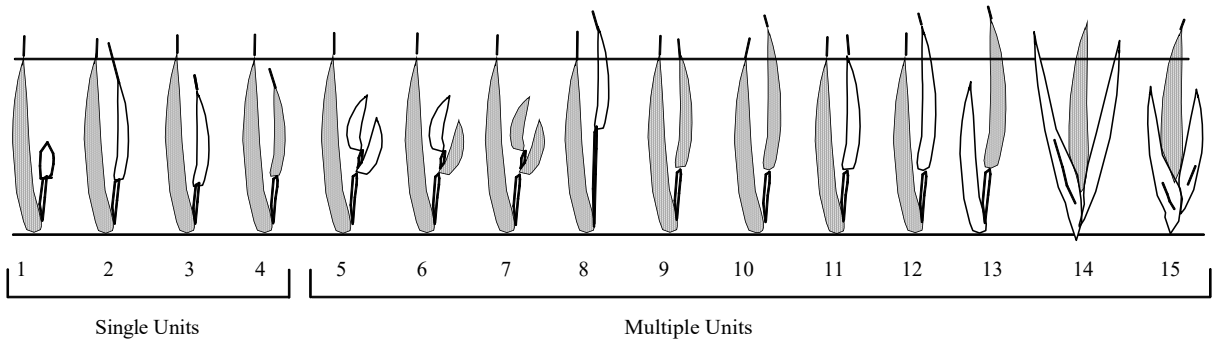


Figure 2 Single and multiple seed units. The shaded portions represent fertile florets and the clear portions represent sterile florets or glumes

3.2.2 Pure Seed

The pure seed must include seed of the crop kind (or kinds) under analysis which must be named in labelling, and includes small, immature, shrivelled, cracked, insect damaged, diseased, sprouted, or otherwise injured seeds, provided that;

a. In the case of pieces of seeds, any piece which is larger than one-half the original size must be considered pure seed except that seeds of the Fabaceae and Brassicaceae with their seedcoats entirely removed must be regarded as inert matter. For separated cotyledons of seeds of the Fabaceae refer to Section 3.2.5.a.iii;

b. Intact seed units (commonly found dispersal units e.g. achenes and similar fruits, schizocarps and mericarps with or without perianth and regardless of whether they contain a true seed) must be considered pure seed unless it is readily apparent that no true seed is present;

   (The term "readily apparent" must be interpreted to mean that the purity analyst must not use a diaphanoscope, stereoscopic microscope, hand lens, pressing or other special equipment or means to detect whether true seeds are present);

c. In the case of the florets and caryopses of Poaceae, pure seed consist of:

   (i). Broken florets or free caryopses, provided they are larger than one-half the original size,

   (ii). Entire florets and one-seeded spikelets with an obvious caryopsis containing endosperm, as determined by the use of slight pressure or by examination over light,

   (iii). Florets of the crop genera *Lolium* and *Festuca* in which the caryopsis is at least one-third the length of the palea as measured from the base of the rachilla,

   (iv). Multiple seed units are left intact in the pure seed portion. In the following genera or species, a sterile floret attached to a fertile floret is not removed but left attached and included in the pure seed fraction: Agropyron cristatum, Agropyron desertorum, Agropyron fragile, Agrostis, Alopecurus arundinaceus, Alopecurus pratensis, Arrhenatherum elatius, Bromus, Dactylis, Elymus trachycaulus, Festuca, Lolium, Phalaris arundinacea, Poa, Puccinellia distans.

   (v). Where the Uniform Blowing Method is used, all material of the kind of seed under analysis which remains in the heavy portion after blowing according to the instructions for that kind of seed, not including:
   - Broken florets or free caryopses which are one-half or less than one-half of the original size,
   - Other crop seeds,
   - Weed seeds,
   - Heavy inert matter,

   (vi). Florets with fungus bodies, such as ergot (*Claviceps purpurea*), entirely enclosed within lemma and palea.

3.2.3 Other Crop Seeds

Seeds of crop kinds listed in the Grade Tables and found as contaminants in a sample must be considered other crop seeds, except that certain seeds and structures as described in Section 3.2.6 must be considered inert matter.

3.2.4 Weed Seeds

Seeds of plants not listed as crop kinds in the Grade Tables must be considered weed seeds, except certain seeds and structures as described in Section 3.2.6 must be considered inert matter. The

classification of seeds as noxious or other weeds must be according to the Weed Seeds Order. Some species, as noted in Section 3.9.3 may have a variable classification under the Seeds Regulations.

Seeds of species not listed as crop kinds in the Grade Tables must be considered crop seeds if they are of the kind named in labelling, but must be considered weed seeds when found as impurities in a sample. (See Section 3.9.8.a)

Structures such as the bulblets of *Poa bulbosa* and *Allium vineale* and tubers of *Cyperus esculentus* must be considered weed seeds.

3.2.5 Inert matter from the crop kind(s) under analysis

a. Pieces of broken or damaged seeds:

(i). One-half the original size or less;

(ii). Seeds of Fabaceae and Brassicaceae with the seedcoats entirely removed;

(iii). Separated cotyledons of Fabaceae, irrespective of whether or not the radicle-plumule axis and/or more than half of the seed coat may be attached;
(iv). Structures defined in Section 3.2.2.b, in which it is readily apparent that no true seed is present.

b. In *Poaceae* when the separation is made using the hand method (i.e. when the Uniform Blowing Method is not prescribed):

(i). Broken florets or free caryopses which are one-half the original size or less;

(ii). Unattached sterile florets, glumes, lemmas, and paleas;

(iii). Florets of *Lolium* and *Festuca* spp. with a caryopsis less than one-third the length of the palea, measured from the base of the rachilla.

c. In *Poaceae* when the separation is made by the Uniform Blowing Method:

(i). In the light portion
- All material **except** other crop seeds as defined in Section 3.2.3 and weed seeds as defined in Section 3.2.4. Seeds of other species of the same genus (e.g. *Poa compressa* in a sample of *Poa pratensis*) must be removed from the light portion and returned to the heavy portion (see Section 3.7.2.b).

(ii). In the heavy portion
- All material **except** pure seed as defined in Section 3.2.2.c.v, other crop seeds as defined in Section 3.2.3, and weed seeds as defined in Section 3.2.4;

3.2.6 Inert matter from weed and other crop plants found as contaminants

All broken seeds, florets or free caryopses which are half or less than one-half the original size, including structures which, by visual examination (including dissection and the use of the diaphanoscope) can be definitely demonstrated as falling within the categories listed below. Doubtful structures must be classed as weed seeds or other crop seeds as the case may be.

a. Seeds and structures of *Poaceae*

(i). Florets or free caryopses, with more than one-half the radicle-plumule axis missing,

(ii). Glumes and empty florets when unattached to fertile florets,

(iii). Attached sterile florets and basal appendages which must be removed from the fertile florets and considered part of the inert matter, **except** in the following cases:
- Attached sterile florets and basal appendages are not removed from: *Agrostis* spp.; *Alopecurus* spp.*; Arrhenatherum elatius; Dactylis glomerata*; *Phalaris arundinacea; Poa* spp.; and
- Attached sterile florets which do not extend to or beyond the tip of the fertile floret are not removed from *Festuca* spp.; *Agropyron cristatum, Agropyron desertorum* or *Agropyron fragile.* The length of an awn must be disregarded when determining the length of a sterile floret.

(iv). Immature florets of *Elymus repens* with a caryopsis less than one-third the length of the palea, measured from the base of the rachilla;

(v). Free undamaged caryopses of *Agropyron, Elymus, Elytrigia, Pascopyrum* or *Pseudoroegneria* spp. 2 mm or less in length;

b. Seeds of families other than *Poaceae*

(i). Seeds devoid of embryo;

(ii). Seeds of *Fabaceae* and *Brassicaceae* with the seed coat entirely removed;

(iii). Separated cotyledons of *Fabaceae*, irrespective of whether or not the *radicle-plumule* axis and/or more than half of the seed coat may be attached;

(iv). Empty fruits (seeds) such as occur in the following plant families: *Asteraceae*, *Convolvulaceae*, *Cyperaceae*, *Polygonaceae*, *Solanaceae*, etc;

(v). Seeds of *Cuscuta* spp. which are either fragile or ashy gray to creamy white in colour;

(vi). Seeds of *Plantago lanceolata* which are black, with no brown colour present, whether shrivelled or plump. The colour of questionable seeds should be determined under a magnification of approximately 10X with strong light;

(vii). Dehulled *Ambrosia* spp. (*involucre* and *pericarp* absent);

(viii). Individual seeds of *Juncus tenuis* or other species of *Juncus* having seeds of a similar size must be considered inert matter. Clusters or capsules of *Juncus* spp. must be left intact, counted and included with the weed seeds; (See Section 3.9.3.f)

(ix). Multiple structures, capsules, pods, heads, etc. are opened, the seeds are removed, and the non-seed material is placed with the inert matter, except as noted in part viii above for *Juncus* spp. (See Section 3.9.8.c)

3.2.7 Inert material other than seeds

The following materials must be classed as **inert matter** in all cases:

a. Nematode galls, including galls enveloped by the lemma and palea of grass florets;

b. Fungus bodies such as ergot (*Claviceps purpurea*), including partially ergotized caryopses and other sclerotia, and smut balls except as defined in Section 3.2.2 c.vi (A partially ergotized caryopsis is any amount of transformation of the caryopsis by the fungus which can be seen by external visual examination and inspection of the internal structure through dissection).

c. Chaff, stems, leaves, stone cells, stones, sand, soil particles, dust, and any other material not seeds;

d. Seeds (crop and weed seeds) in which larvae occupy one-half or more of the seed unit.

3.2.8 "On-the-line"

In the analysis of a sample of seed, the phrase "on the line" means that the number of foreign seeds of a particular category (such as primary noxious weed seeds), or the percentage by weight of a particular component (such as other crop seeds) found in the quantity analysed, lies within the limits given in the appropriate table of checking limits (Sections 3.5.3 and 3.6.3) between which a check test is required for the grade line in question. The "line" is the maximum number of foreign seeds of a particular category, or the maximum or minimum percentage of a particular component, permitted in any given grade by the Tables of Grade Standards, Schedule I, Seeds Regulations.

3.2.9 Unit Weight

Refers to the weight upon which a grade standard for numbers of impurities is based i.e. number of impurities per 25g, 500g or 1kg.

3.3 WORKING SAMPLE

Working samples of the required size are drawn from the submitted sample using the procedures outlined in Section 2.2. Weights of working samples are determined from Section 2.3.4 Table 1.

3.3.1 Weighing

The following table states the minimum number of decimal places required:

a. when weighing to obtain a working sample(s) (e.g. percent test, 1st , 2nd or 3rd quantities),

b. when weighing any component part of a working sample,

c. in order to calculate to one decimal place.

Weight of Working Sample in grams	Weigh the working sample and it's components to the following number of decimal places
less than 1	4
1 to 9.999	3
10 to 99.99	2
100 to 999.9	1
1000 or more	0

3.4 GENERAL PROCEDURE FOR PURITY ANALYSIS

The analysis of the working sample follows the procedure outlined below (Note: **"Table"** refers to the tables of this manual; **"Grade Table"** refers to the Tables of Grade Standards, Schedule I, Seeds Regulations):

a. Consult the appropriate Grade Table for the kind of seed under analysis to determine the standards for which analytical data are required (e.g. numbers of weed seeds, percentage pure seed, percentage other crop seeds, etc.). For single species or seed mixtures composed of or, containing kinds not listed in the Grade Tables see Section 3.4.1.

b. Determine the size of the working sample portions from Columns 3, 4, 5 and 6 of Section 2.3.4 Table 1. For single species or seed mixtures composed of or, containing kinds not listed in the Grade Tables see Section 2.3.5.

c. Draw the working sample portions from the submitted sample as described in Section 2.3 and 3.3. A single quantity equal to the combined quantities of columns 3, 4 and 5 of Section 2.3.4 Table 1 may be drawn if a full analysis is to be conducted as per Section 3.6.1.

d. If required by the Grade Table, conduct any tests for percentage by weight, as described in Section 3.5.

e. When a Grade Table standard for other non-Brassica crop seeds is stated as numbers per unit weight and the kind under analysis is difficult to separate from similar species (i.e. Section 3.8.2, 3.8.6 and 3.8.7) a minimum of one-fifth of the total working sample weight stated in Columns 3, 4 and 5 of Section 2.3.4 Table 1 is required to be examined for similar species (e.g. Foundation status Elymus trachycaulus, Slender wheatgrass).

f. Conduct an analysis for determination of numbers of impurities per unit weight (i.e. per 25g, per 500g or per kg, as determined from the headings of the appropriate Grade Table), as described in Section 3.6.

g. Identify and classify all impurities found during the analyses conducted in (d) and (e) above. For classification of impurities, refer to the Seeds Regulations, the Weed Seeds Order and Section 3.9.3.

h. Report the results on a worksheet and/or report of analysis, as described in Section 1.0 and 3.9.

3.4.1 Procedure for Analysis of kinds not listed in the Seeds Regulations, including mixtures of seeds

a. Grade Standards

The *Seeds Regulations* (Section 6(2)) require that seed of kinds not listed in Schedule I, must meet the minimum per unit weight purity standards and where applicable, the percent weed seeds and/or other crop seeds standards* of the following Grade Tables. The end use of the product, when known, takes precedence when deciding which Grade Table to use.

Grade Standards		
End use of seed or kind/species	**Number of seeds per gram**	**Grade Table**
Field crops, other than grasses	15 or fewer 16 to 50 51 to 250 251 to 600 601 or more	V II IV VIII IX
Any grass (*Poaceae*)	1500 or fewer 1501 or more	XI XII
Any seed or mixture for land reclamation soil conservation, green cover, wildlife grazing or habitat, wetland restoration and similar purposes	All	XIII
Herbs and vegetables	999 or fewer 1000 or more	XX XII
Wildflower mixtures and similar products intended for landscape gardening use	All	XV

* **It is not required for kinds not listed in Schedule I to meet the standards for percent pure seed or percent pure living seed**.

Hemp Cannabis sativa L. subsp.sativa is listed in Grade Table IV, therefore seed samples of Industrial Hemp must meet the standards in that table. All other cannabis must meet the minimum per unit weight purity standards in Grade Table IV.

b. Analysis of single species.

When determining the number of other species per unit weight, use the quantity given in Section 2.3.5.b, column 3 (i.e. 2500 seed portion) to conduct a full analysis (See Section 3.6.1). Multiply other weeds and other crops up to the weight in terms of the unit weight as set out in the appropriategrade table (3.9.2.b). Use the quantity given in column 2 (i.e. 25000 seed portion) to analyse for all Prohibited, Primary and Secondary noxious weeds. Retrieve and report impurities as required by the Seeds Regulations and the appropriate Grade Table as determined in Section 3.4.1.a. If it is required to determine percentage by weight for any factor, use the percentage test working sample size given in Section 2.3.5.b, column 3 and follow the procedure of Section 3.4 and 3.5.

c. Analysis of mixtures of seeds containing:

- kinds not listed in the Grade Tables or
- kinds not listed in the Grade Tables and listed in the Grade Tables.

(i). Grade Standards –
The minimum grade standards as described in Section 3.4.1.a. apply.

(ii). Analysis. When determining the number of other species per unit weight, use the quantity determined by Section 2.3.5.c.iii, to conduct a full analysis (see Section 3.6.1). Retrieve and report impurities as required by the Seeds Regulations and the appropriate Grade Table as determined in Section 3.4.1.a. If it is required to determine percentage by weight for any factor, use the percentage test working sample as estimated by using the procedure in Section 2.3.5.b and follow the procedures of Section 3.4 and 3.5.

3.5 DETERMINATION OF PERCENTAGE BY WEIGHT

3.5.1 General Procedure

a. Obtain the required quantity of seed as specified in Column 6 of Section 2.3.4 Table 1 for determination of percentage by weight, following the directions given in Section 2.3.2. For mixtures, see also Section 2.3.3. See Section 3.4.1 for the analysis of single species or seed mixtures composed of or, containing kinds not listed in the Grade Tables.

b. Record the initial weight of the quantity to be analysed. After analysis, the weights of all the components of the separation must be added together and recorded. If the sum of the weights of all the components of the separation differs from the initial weight by more than 5%, a retest must be conducted. The result of the retest must be reported.

c. Separate the quantity into the fractions which will be required in grading or labelling (in the case of mixtures). The number of fractions and their contents will depend on the requirements of the Grade Tables, as described in Section 3.5.2.

d. Use the checking limits of Table 2, Section 3.5.3.b, to compare the percentages obtained to the grade standards of the appropriate Grade Table. Perform a check test on the quantity given in Section 2.3.4 Column 6 of Table 1 if "on-the-line". If the difference between the original and check tests exceeds the tolerance given in Section 3.5.3.c, Table 3, conduct a third test and average the results which are compatible according to Table 3. If the third test falls between the first two and is compatible with both, then report the average of the three tests. If there are no compatible results after three check tests, analyse another working sample (not more than four tests in total). Report the weighted average of the check tests for which the highest and lowest results do not differ by more than twice the tolerance stated in Table 3, unless it is apparent that one or more of these results are due to an error and not to random sample variation. In that case, discard the test(s) with the errors. If no pair of results is within tolerance, it is advisable to find the cause of the variation

e. Record the name and number of any impurities which belong to one of the numerical grading categories (e.g. noxious weed seeds). These numbers are to be combined with those found in the portion from which the percentage test quantity was taken

f. Continue with Section 3.4.e and 3.4.f of the general procedure to determine numbers of impurities per unit weight

3.5.2 Specific Grade Table Requirements for Percentages by Weight

a. Grade Table III (Cereal Mixtures)
Separate into (i) the kinds which are permitted under Grade Table III and (ii) everything else (i.e. incidental crop and weed seeds and inert matter). Particles which are readily identifiable as belonging to a component species are included in the separation for that component, regardless of whether or not they are pure seed or include a pure seed unit. Only loose chaff, pieces of leaves or stems, dirt, etc. are separated as inert matter. Only the percentage by weight of each of the crop kinds permitted under Grade Table III are reported.

b. Grade Tables VIII, IX and X (Alfalfa, clovers, etc.)
Separate into **two** fractions, namely (i) other crop seeds and (ii) everything else. For Grade Table IX separate into **three** fractions (i) other crop seeds (ii) ergot and/or sclerotia bodies and (iii) everything else. Note that sweet clover, *Brassica* species and *Sinapis alba* are not included in the other crop fraction. Weigh and report the percentage of the other crop seeds and list the kinds of other crop seeds present. If it is clearly evident that the amount of other crop seeds could not be a deciding factor in establishing the grade this test need not be conducted. In this case it is necessary to list the kinds of other crop seeds present and report the occurrence of other crop seeds as being less than the maximum percentage allowed as if the grade were to be established on the basis of only the other crop seeds (e.g. "less than 2%"). Use this same procedure to determine and report the percentage by weight of ergot and sclerotia in crop kinds of Grade Table IX.

c. Grade Table XI (Large grasses)
Separate into **four** fractions following the pure seed definitions: (i) pure seed, (ii) other crop seeds, (iii) ergot and (iv) everything else. Note that sweet clover, *Brassica* species and *Sinapis alba* are not included in the other crop fraction. Weigh and report the percentage pure seed and percentage other crop seeds. If it is clearly evident that the amount of other crop seeds could not be a deciding factor in establishing the grade, these may be combined with the "everything else" fraction for weighing and reported as described for clovers above. Use this same procedure to determine and report the percentage by weight of ergot in crop kinds in Grade Table XI.

d. Grade Table XII (Small grasses)
Separate into **five** fractions following the pure seed definitions: (i) pure seed, (ii) weed seeds (all classes), (iii) other crop seeds, (iv) ergot and (v) inert matter. Weigh and report the percentage of each of these fractions. For samples of *Poa*, see also Section 3.8.4 and for samples of *Agrostis* see also Section 3.8.3. Ensure there is sufficient pure seed of the species under analysis for the germination test.

e. Grade Table XIII (Forage Mixtures)
Separate into (i) the kinds which are permitted under Grade Table XIII, (ii) other crop seeds, (iii) sweet clover, (iv) ergot and (v) everything else. Note that *Brassica* species and *Sinapis alba* are not included in the other crop fraction. Sweet clover present at a rate of 1% or more is considered one of the components of the mixture. If it is clearly evident that sweet clover is present at a rate of less than 1% of the mixture, it may be combined with the "everything else" fraction for weighing, but their numbers must be included with those from the determination of impurities by numbers (Section 3.6). If it is clearly evident that the percentage of other crop seeds could not be a deciding factor in establishing the grade, these may be combined with the "everything else" fraction for weighing and reported as described for clovers, above. Particles which are readily identifiable as belonging to a component species are included in the separation for that component, regardless of whether or not they are pure seed or include a pure seed unit. Only loose chaff, pieces of leaves or stems, dirt, etc. are separated as inert matter. Weigh and report the percentage of each of the kinds which are permitted under Grade Table XIII, other crop seeds, ergot and sweet clover if over 1%. See below for examples of reporting procedures.

Separate sufficient pure seeds of each component species for a germination test. See Section 4.4.2 for the number of seeds required for germination testing.

See the Seeds Regulations for forage mixture labelling requirements.

Example - reporting procedures for forage mixtures.

(i). Forage Mixture with more than 1% sweet clover.

In "Remarks"		Sweet clover -- In "Other Crop Seeds"	
Timothy	62.5%	Orchardgrass	(1.0%)
Alsike	33.0%	Alfalfa	
Sweet clover	1.5%	Kentucky bluegrass	
		Canada bluegrass	
		Total	2.0%

(ii). Forage Mixture with less than 1% sweet clover.

In "Remarks"		Sweet clover 19/25g. In "Other Crop Seeds"	
Timothy	63.5%	Orchardgrass	(1.0%)
Alsike	33.0%	Alfalfa	
		Kentucky bluegrass	
		Canada bluegrass	
		Total	2.0%

f. Grade Table XIV (Lawn and turf mixtures)
Separate into the following fractions using the pure seed definitions:

(i). Pure seed of each kind permitted* under Grade Table XIV, i.e:
- each grass listed in Groups A and B of Part II;
- any other grass to be named in labelling (Group C of Part II)

(ii). White clover (column V of Part I);

(iii). Bromegrass, orchardgrass and tall fescue (column VI of Part I)

(iv). Other crop seeds, i.e. all kinds not included in i, ii or iii above;

(v). Weed seeds;

(vi). Ergot; and

(vii). Inert.

* Include Timothy, tall fescue, perennial ryegrass and annual ryegrass as components only if named in labelling since the analyst cannot verify dwarf or turf types or differentiate with accuracy between perennial and annual ryegrasses. When a mixture contains components which cannot be accurately separated, the analyst need not attempt to do so. In this case combine all seeds of similar species and report the generic name (e.g. *Festuca* spp. or Fescue). The reporting procedure described in Section 3.9.8.e may also be used. If an estimate of percentages of similar species is required, an appropriate methodology should be used (e.g. fluorescence test as described by AOSA).

Weigh and report the name and percentage pure seed of each kind separated under part (i), including any that are "trace". The total of these percentages is reported as the percentage pure seed of the mixture. If white clover is to be named in labelling, include the white clover in the listing with the other components and include it in the percentage pure seed. Weigh and report in the appropriate spaces the fractions separated under parts (ii) through (vii). If “on the line" for the percentage of any component(s), only the percentage of the component(s) being checked needs to be determined (e.g. % pure seed, % weed seeds, etc). See below for an example which illustrates the reporting procedure.

For mixtures containing kinds listed in Section 3.7 as requiring use of the Uniform Blowing Method, see Section 3.7.5. See Section 3.8.3 for mixtures containing *Agrostis* species and Section 3.8.4 for mixtures containing *Poa* species.

Separate sufficient pure seeds of each component species for a germination test. See Section 4.4.2 for the number of seeds required for germination testing.

For regulations concerning the labelling of component percentages, see the Seeds Regulations.

Example reporting for a lawn mixture to be labelled with the following components: Red fescue, ryegrass and Kentucky bluegrass. It is not required to report the figures given in brackets. Note that crested wheatgrass is listed with the components even though it is not going to be labelled as a component, because it is named in Part II of Grade Table XIV. The percentage “Other crop seed” has been marked as “--” because there is more than one “other crop” category (i.e. white clover, bromegrass, etc. and other kinds). The actual percentage other kinds has been reported as 1.6%, but it could have been reported as “less than 2%”.

In "Other Crop Seeds":			**In Percentages Section:**	
White clover	(0.6%)	Less than 5%	Pure Seed	94.6%
			Other crop seed	--
Bromegrass	(0.2%)		Weed seed	0.1%
Tall Fescue	(0.5%)	Less than 1%	Inert	2.4%
Birds-foot trefoil	(0.3%)		**In "Remarks":**	
Alsike clover	(0.5%)			
Red clover	(0.4%)		Red fescue	43.9%
Meadow fescue	(0.4%)	1.6%	Ryegrass	28.7%
			Kentucky bluegrass	19.3%
			Crested wheatgrass	2.7%
			Total	94.6%

g. Grade Table XV (Ground cover mixtures)

Separate into the following fractions using the pure seed definitions: (i) pure seed, (ii) other kinds, (iii) weed seeds and (iv) inert. For purposes of Grade Table XV, species in ground cover mixtures are to be classified according to the following definitions:

Pure Seed - Includes seeds of all species identified by the packager as components of the mixture.

Other kinds - Includes seeds of species listed in Schedule I of the Seeds Regulations but not identified by the packager as components of the mixture.

Weed seeds - Includes seeds of species not listed in Schedule I of the Seeds Regulations and not identified by the packager as components of the mixture.

Weigh and report under "Remarks" the name and percentage pure seed of each kind separated under part (i), including any that are "trace". The total of these percentages is reported as the percentage pure seed of the mixture. Weigh and report in the appropriate spaces the percentages of other crop seeds and total weed seeds.

For mixtures containing kinds listed in Section 3.7 as requiring use of the Uniform Blowing Method, see Section 3.7.5. See Section 3.8.3 for mixtures containing *Agrostis* species, and Section 3.8.4 for mixtures containing *Poa* species.

If "on the line" for any percentage, only the percentage of the component being checked needs to be determined (i.e. % pure seed, % weed seeds or % other kinds).

Separate sufficient pure seeds of each component species for a germination test. See Section 4.4.2 for the number of seeds required for germination testing.

3.5.3 Table of Checking Limits for Percentages

a. Use of Section 3.5.3.b. Table 2 and Section 3.5.3.c. Table 3

Table 2 is used to decide whether a "check" test is required for a percentage determination. In order to use this table, the analyst must consult the appropriate Grade Table for the kind of seed under analysis to determine the grade maximum or minimum for each grading factor which is based on a percentage. Table 3 is used to determine if a check test is compatible with the first test and the two can be averaged.

To use Table 2 (3.5.3.b.):

(i). Determine which columns of lower and upper limits will be used, based on whether the seed is chaffy or non-chaffy. Seeds of the following genera are to be regarded as chaffy: *Agropyron, Agrostis, Alopecurus, Arrhenatherum, Bromus, Cynosurus, Dactylis, Elymus, Elytrigia, Festuca, Leymus, Lolium, Panicum, Poa, Pascopyrum, Psathyrostachys, Pseudoroegneria, Puccinellia.*

(ii). Enter the table at the percentage "specification" given in the Grade Table. For example, the minimum percent pure seed for Canada Certified No.1 bluegrass (Grade Table XII) is 85%.

(iii). Move across to the appropriate column of lower and upper limits. Since bluegrass is considered chaffy, the second pair of limits would be used.

(iv). Read the lower and upper limits. For the bluegrass example, the lower and upper limits would be 83.62% and 86.38%, respectively. If the percentage determined in the analysis lies on or between the limits, it is "on-the-line" for the grade line in question and a "check" test must be performed on an equivalent quantity of seed. If the percentage lies outside the limits, then it is not necessary to conduct a check test.

To use Table 3 (3.5.3.c.):

(i). Enter the table at the average percentage determined from the two working samples. For example, if the pure seed test of a bluegrass sample is 84.1% and the check test is 85.9%, the average would be 85.0% and the table would be entered at the 84.00 - 85.99 row.

(ii). Move across to the appropriate column of tolerances.

(iii). Read the tolerance. If the range between the two tests being compared is equal to or less than the tolerance, then the percentages are averaged. If the range exceeds this tolerance, then the tests cannot be averaged (see Section 3.5.1.e). For the bluegrass example, the tolerance from the chaffy column is 2.62%. The difference between the two tests is 1.8% which is less then the tolerance, therefore the average of 85.0% is reported.

3.5.3.b. Table 2. Checking limits for percentage of pure seed, inert matter, weed seeds, sweet clover, other crop seeds, ergot and sclerotia.

Specification given in the Grade Table (%)	Checking Limits (%)			
	Non-chaffy Seed		Chaffy Seed	
	Lower	Upper	Lower	Upper
0.1	- *	0.22	-	0.25
0.2	0.03	0.37	-	0.40
0.3	0.10	0.50	0.06	0.54
0.4	0.17	0.63	0.13	0.67
0.5	0.24	0.76	0.20	0.80
0.8	0.49	1.11	0.42	1.18
1	0.65	1.35	0.58	1.42
1.5	1.10	1.90	1.03	1.97
2	1.52	2.48	1.43	2.57
3	2.42	3.58	2.31	3.69
4	3.35	4.65	3.22	4.78
5	4.28	5.72	4.14	5.86
6	5.22	6.78	5.07	6.93
7	6.16	7.84	6.01	7.99
8	7.10	8.90	6.95	9.05
9	8.06	9.94	7.89	10.11
10	9.01	10.99	8.84	11.16
15	13.82	16.18	13.62	16.38
20	18.69	21.31	18.45	21.55
25	23.57	26.43	23.33	26.67
30	28.49	31.51	28.23	31.77
35	33.43	36.57	33.16	36.84
40	38.38	41.62	38.10	41.90
45	43.36	46.64	43.08	46.92
50	48.35	51.65	48.06	51.94
55	53.36	56.64	53.08	56.92
60	58.38	61.62	58.10	61.90
65	63.43	66.57	63.16	66.84
70	68.49	71.51	68.23	71.77
75	73.57	76.43	73.33	76.67
80	78.69	81.31	78.45	81.55
85	83.82	86.18	83.62	86.38
90	89.01	90.99	88.84	91.16
95	94.28	95.72	94.14	95.86
96	95.35	96.65	95.22	96.78
97	96.42	97.58	96.31	97.69
98	97.52	98.48	97.43	98.57
99	98.65	99.35	98.58	99.42

"-" in a checking limit column indicates that the analysis must be continued into the next portion.

Tolerances are calculated from Table P20, Handbook of Tolerances, S.R. Miles, Proceedings of the International Seed Testing Association Vol. 28, No. 3, 1963. Formula used: (20%) Tol.=(5%)Tol./1.96 x 1.28 (two-way test at 0.20 probability level).

3.5.3.c. Table 3. Tolerances for comparing percentage of pure seed, inert matter, weed seeds, sweet clover other crop seeds, ergot and sclerotia, to determine if two tests are compatible and may be averaged (See Section 3.5.1.e).

Average of 2 Estimates		Tolerance (%)	
		Non-chaffy seed	Chaffy seed
99.95-100.00	**0.00-0.04**	0.14	0.16
99.90-99.94	**0.05-0.09**	0.23	0.24
99.85-99.89	**0.10-0.14**	0.28	0.30
99.80-99.84	**0.15-0.19**	0.33	0.35
99.75-99.79	**0.20-0.24**	0.36	0.39
99.70-99.74	**0.25-0.29**	0.39	0.42
99.65-99.69	**0.30-0.34**	0.43	0.46
99.60-99.64	**0.35-0.39**	0.46	0.49
99.55-99.59	**0.40-0.44**	0.48	0.52
99.50-99.54	**0.45-0.49**	0.51	0.54
99.40-99.49	**0.50-0.59**	0.54	0.58
99.30-99.39	**0.60-0.69**	0.59	0.63
99.20-99.29	**0.70-0.79**	0.63	0.67
99.10-99.19	**0.80-0.89**	0.67	0.71
99.00-99.09	**0.90-0.99**	0.71	0.75
98.75-98.99	**1.00-1.24**	0.76	0.81
98.50-98.74	**1.25-1.49**	0.84	0.89
98.25-98.49	**1.50-1.74**	0.91	0.97
98.00-98.24	**1.75-1.99**	0.97	1.04
97.75-97.99	**2.00-2.24**	1.02	1.09
97.50-97.74	**2.25-2.49**	1.08	1.15
97.25-97.49	**2.50-2.74**	1.13	1.20
97.00-97.24	**2.75-2.99**	1.18	1.26
96.50-96.99	**3.00-3.49**	1.25	1.33
96.00-96.49	**3.50-3.99**	1.33	1.41
95.50-95.99	**4.00-4.49**	1.41	1.5
95.00-95.49	**4.50-4.99**	1.48	1.57
94.00-94.99	**5.00-5.99**	1.59	1.68
93.00-93.99	**6.00-6.99**	1.72	1.81
92.00-92.99	**7.00-7.99**	1.83	1.93
91.00-91.99	**8.00-8.99**	1.94	2.05
90.00-90.99	**9.00-9.99**	2.04	2.15
88.00-89.99	**10.00-11.99**	2.18	2.30
86.00-87.99	**12.00-13.99**	2.34	2.47
84.00-85.99	**14.00-15.99**	2.49	2.62
82.00-83.99	**16.00-1799**	2.61	2.76
80.00-81.99	**18.00-19.99**	2.73	2.88
78.00-79.99	**20.00-21.99**	2.83	2.99
76.00-77.99	**22.00-23.99**	2.93	3.09
74.00-75.99	**24.00-25.99**	3.01	3.18
72.00-73.99	**26.00-27.99**	3.09	3.26
70.00-71.99	**28.00-29.99**	3.16	3.33
65.00-69.99	**30.00-34.99**	3.26	3.44
60.00-64.99	**35.00-39.99**	3.37	3.55
50.00-59.99	**40.00-49.99**	3.46	3.65

Tolerances are extracted from Table P11, *Handbook of Tolerances,* S.R. Miles, Proceedings of the International Seed Testing Association Vol. 28, No.3, 1963. (Two-way test at 0.05 probability level)

3.6 DETERMINATION OF NUMBERS PER UNIT WEIGHT

The laboratory may choose either to conduct a full analysis of the working sample, or to follow a sequential analysis procedure, either of which will give the same grading decision. While the sequential analysis procedure is more complex than the full analysis procedure, in some cases it is a more efficient analytical method.

3.6.1 Full Analysis

Analyse the combined weights of Columns 3, 4 and 5 of Table 1 Section 2.3.4 for all of the impurities which are listed as grading factors under the Grade Table for the crop kind under analysis and the Seeds Regulations. At the conclusion of the analysis, continue the steps as outlined in Section 3.4.f.

3.6.2 Sequential Analysis

The purpose of using a sequential analysis procedure is to allow the possibility of reducing the quantity of seed that must be examined for one or more impurities. The working sample is divided into smaller portions of the sizes given in Columns 3, 4 and 5 of Section 2.3.4 Table 1. These portions are examined sequentially, one after the other, basing a decision as to whether the next portion needs to be examined on the numbers of impurities found in the total quantity analysed up to that point. It may be possible to stop searching for certain impurities, or if impurities are excessive, it may be possible to terminate the analysis before the full working sample has been analysed. A table of checking limits in Section 3.6.3.b. Table 4 is used in making this decision.

To conduct a sequential analysis:

a. Follow the sequential analysis steps as given in the Key to Column Headings for Table 1 (Section 2.3.4). Be certain to include the counts of impurities found in any percentage by weight tests with impurities found in the portion from which the percentage test quantity was drawn.

b. At the end of the analysis of each portion of seed, use the checking limits of Table 4, Section 3.6.3.b, to compare the numbers of impurities obtained, in the quantity analysed, to the grade standards of the appropriate Grade Table. The analysis is continued on the next portion as directed in the Key to Column Headings. The table of checking limits is used after analysis of the first and second portions only. The table is not consulted at the end of the third portion, because at this point the full analysis has been completed.

c. At the conclusion of the analysis, complete the steps outlined in the general procedure, Section 3.4.f.

3.6.3 Table of Checking Limits for Numbers

a. Use of Table 4

Table 4 is used during a sequential analysis, for deciding whether analysis of an additional quantity of seed is required. See the definition of "on the line", Section 3.2.8. In order to use this Table, the analyst must consult the appropriate Grade Table for the kind of seed under analysis to determine (a) the quantity of seed on which the grade standard is based, and (b) the grade maximum for each grading factor. For example, for Canada Certified No. 1 alsike (Grade Table IX), the maximum number of primary plus secondary noxious weed seeds allowed is 5 per 25g ("5" is the grade maximum; "25g" is the weight on which the standard is based). To use Table 4:

(i). Determine which columns of upper and lower limits will be used. First, find the row in the column headed "Grade maximum stated on basis of" which corresponds to the weight upon which the standard is based in the Grade Table. For the alsike example, the standard is based on the number of seeds per 25g; therefore the first row would be used.
Next, move across this row to the weight which corresponds to the total quantity of seed which has been analysed up to that point. For alsike, a total of 12.5g would have been analysed at the end of the second portion, so move across the top row to the "12.5g" column.

(ii). Enter the Table at the appropriate grade maximum for the impurity under consideration. For the alsike example, the maximum primary plus secondary noxious weeds are 5 per 25g, so move down the "Grade maximum" column to the number "5".

(iii). Move across to the appropriate pair of lower and upper limit columns as determined in step i. For the alsike example, it will be the "12.5g 250g 500g" column.

(iv). Read the upper and lower limits. For the alsike example, with a grade maximum of 5, the lower limit is 1 and the upper limit is 5. If the number of the impurity found during the analysis up to that point lies on or between the limits, it is "on-the-line" for the grade line in question and the next portion must be analysed for that impurity. If the number lies below the lower limit, then it is not necessary to analyse the next portion for that impurity. If the number lies above the upper limit then the sample will be downgraded (or rejected) because of that impurity. In this case the checking limits table must be re-entered at the grade maximum for the lower grade to determine if the impurity is "on-the-line" for that grade.

3.6.3.b. see Table 4 on next page.

3.6.3.b.Table 4. Checking limits for numbers of impurities in quantity analysed in tests for determination of grade.

Grade maximum stated on basis of:	Quantity Analysed																	
25g	250g		150g		125g		75g		50g		25g		12.5g		6.25g		3.13g	
500g	n/a[1]		n/a		n/a		n/a		n/a		n/a		250g		125g		n/a	
1kg	n/a		n/a		n/a		n/a		n/a		n/a		500g		250g		125g	
Grade maximum	L[2]	U[3]	L	U	L	U	L	U	L	U	L	U	L	U	L	U	L	U
0	0	1	0	1	0	1	0	1	-[4]	-	-	-	-	-	-	-	-	-
0.5	1	10	0	7	0	6	0	5	0	2	0	1	-	-	-	-	-	-
1	4	17	1	11	1	10	0	7	0	4	0	2	0	1	-	-	-	-
2	12	29	6	19	4	17	1	11	2	7	0	4	0	2	0	1	-	-
3	20	40	10	26	8	23	3	15	3	9	1	5	0	3	0	1	-	-
4	29	52	15	33	12	29	6	19	5	12	2	7	0	4	0	2	0	1
5	38	63	20	40	16	35	8	23	6	14	2	8	1	5	0	2	0	1
6	47	74	25	47	20	40	10	26	8	17	3	9	1	5	0	3	0	1
7	55	85	31	54	25	46	13	30	9	19	4	10	1	6	0	3	0	1
8	65	96	36	61	29	52	15	33	11	21	5	12	2	7	0	4	0	2
9	74	107	41	67	33	57	18	37	13	24	6	13	2	7	0	4	0	2
10	83	118	47	74	38	63	20	40	15	26	6	14	2	8	1	5	0	2
12	101	139	57	87	47	74	25	47	18	31	8	17	3	9	1	5	0	3
13	110	150	63	94	51	80	28	51	20	33	8	18	3	9	1	5	0	3
14	120	161	68	100	55	85	31	54	21	35	9	19	4	10	1	6	0	3
15	129	172	74	107	60	91	33	57	23	37	10	20	4	11	1	6	0	3
16	138	182	79	113	65	96	36	61	25	40	11	21	5	12	2	7	0	4
20	176	225	101	139	83	118	47	74	32	48	15	26	6	14	2	8	1	5
22	195	246	112	152	92	129	52	81	35	53	17	28	7	16	2	9	1	5
24	214	267	124	165	101	139	57	87	40	57	18	30	7	16	3	9	1	5
25	223	277	129	172	106	145	60	91	41	60	19	32	8	17	3	9	1	5
30	271	330	157	203	129	172	74	107	50	70	23	37	10	20	4	11	1	6
35	318	382	185	235	152	198	87	123	59	81	28	43	12	23	5	12	2	7
40	366	434	214	267	176	225	101	139	68	92	32	48	15	26	6	14	2	8
44	405	476	236	292	195	246	112	152	76	101	35	53	17	28	7	16	2	9
50	462	538	271	330	223	277	129	172	87	114	41	60	19	32	8	17	3	9
60	559	642	328	393	271	330	157	203	105	135	50	70	23	37	10	20	4	11
70	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	124	156	59	81	28	43	12	23	5	12
75	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	134	167	64	87	30	46	13	24	6	13
80	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	143	177	68	92	32	48	15	26	6	14
90	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	162	198	78	103	36	54	17	29	7	16
100	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	181	219	87	114	41	60	19	32	8	17
120	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	219	261	105	135	50	70	23	37	10	20
125	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	229	272	110	140	52	73	25	39	11	21
150	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	277	324	134	167	64	87	30	46	13	24
180	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	334	386	162	198	78	103	36	54	17	29
200	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	373	427	181	219	87	114	41	60	19	32
250	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	466	534	229	272	110	140	52	73	24	39
300	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	567	633	277	324	134	167	64	87	30	46

[1] n/a denotes "not applicable"

[2] L - lower limit

[3] U - upper limit

[4] "-" in a checking limit column indicates that the analysis must be continued into the next portion.

Adapted from tables in *Statistical Methods for Seed Analysts*, 1942. C.W. Leggatt. Published by the Plant Products Division, Canada Department of Agriculture, 1960, and from Table F3, Handbook of Tolerances, S.R. Miles, Proceedings of the International Seed Testing Association Vol. 28, No.3, 1963.

3.7 UNIFORM BLOWING METHOD

3.7.1 General Procedure

The Uniform Blowing Method must be used as part of the procedure for determination of percentage pure seed for *Dactylis glomerata* and certain species of *Poa* and *Agrostis* as listed below. The quantity of seed specified in Column 6 of Table 1 Section 2.3.4 is blown for precisely 3 minutes at the setting for the individual machine, multiplied by the appropriate factor for the species, where applicable. The setting (or Uniform Blowing Point, UBP) for Kentucky bluegrass or orchardgrass must be pre-determined by using the 1g *Poa pratensis* or 3g *Dactylis glomerata* calibration sample, respectively, available from the USDA Seed Regulatory and Testing Branch. When the blower is calibrated for *Dactylis glomerata*, the large tube must be used. For the species listed below which require use of a factor to determine the setting, the *Poa pratensis* UBP is to be used in the calculation. The instructions in the AOSA Rules, Volume 2, *The Uniform Blowing Procedure*, must be followed for calibration and use of the blower.

After blowing, there will be two fractions:

a. **Light fraction**. The light fraction is the material which has blown over into the receptacle and is considered inert matter except for seeds of weeds and other crops (including those of the same genus as the kind under analysis).

b. **Heavy fraction**. The heavy fraction is the material which has remained in the cup after blowing and is considered pure seed (multiple seed units are left intact) except any heavy inert matter (e.g. plant material, stones), weed seeds and other crop seeds (including those of the same genus as the kind under analysis).

The pure seed from the heavy fraction and the combined like components (i.e. other crop seeds, weed seeds and inert matter) from the light and heavy fractions are weighed. Percentages as required by the appropriate Grade Table are determined from these weights.

3.7.2 Procedure for Poa species

a. Blow 1g of seed for exactly 3 minutes at the appropriate blowing point as follows:

   (i). *Poa pratensis* - UBP

   (ii). *Poa compressa* - UBP X 0.97

   (iii). *Poa trivialis* - UBP X 0.82

b. Separate the components of the heavy and light fraction as described in the general procedure (Section 3.7.1). All *Poa* other crop seeds which are not inert are removed from the light fraction and returned to the heavy fraction. Weigh and calculate the percentage for: "pure bluegrass spp." and the combined like components for other crop seeds (other than *Poa* spp.), weed seeds and inert matter. Examine 400 seeds or 0.15g from the "pure bluegrass" component for the presence of other species of *Poa* and calculate the final percentages following the procedure of Section 3.8.4.

c. Mixtures containing two or more species of *Poa*. For a mixture containing *Poa pratensis* and *Poa trivialis*, blow the total bluegrass component at the UBP, then blow the light fraction at UBP X 0.82. From the heavy fraction of the second blowing, separate the two species by hand. Seeds of *Poa pratensis* from this fraction will be placed with the inert. Seeds of *Poa trivialis* will be placed with the "pure bluegrass" component. Follow a similar procedure for any mixture containing *P. compressa*, but blow at UBP X 0.97. If the mixture contains species of *Poa* for which there is no blowing point, the light fraction must be searched for seeds of these species, and the seeds assessed by hand to determine if they meet the pure seed definitions.

3.7.3 Procedure for *Agrostis* species

When analysing *Agrostis*, the attached empty glumes must not be removed.

a. Blow 0.5g of seed for 3 minutes at the appropriate blowing point as follows:

   (i). *Agrostis gigantea* - UBP X 0.68.

   (ii). Other species of *Agrostis*:
   1. Blow 0.5g of seed for 3 minutes at UBP X 0.49. The fraction blown over is put aside as inert matter.
   2. Blow the heavy fraction from (1) for 3 minutes at UBP X 0.65. From the light fraction, separate the pure seed by hand. From the heavy fraction, separate the inert matter by hand.
   3. Combine the inert matter from the light fractions of (1) and (2) with the heavy inert from (2) to determine the percentage of inert matter. Combine the two pure seed fractions to determine the percentage of pure seed.

b. For additional procedures to be followed when testing *Agrostis*, See Section 3.8.3.

3.7.4 Procedure for Dactylis glomerata

a. Blow 3g of seed for exactly 3 minutes at the appropriate blowing point as determined by calibrating the blower with the AOSA orchardgrass calibration sample.

b. Upon request, the percentage by weight of multiple seed units found in a sample can be reported under other determinations on the report of analysis.

3.7.5 Procedure for components of mixtures

In general, the weight of a component in a mixture working sample will be less than the prescribed weight for that species as given in Column 6 of Table 1 Section 2.3.4. Therefore, except as noted in Section 3.7.2.c, the uniform blowing method should be used only as an aid in determining the percentage pure seed of *Agrostis*, *Dactylis* or *Poa* when these are present as components of lawn or turf or ground cover mixtures. If the blower is used when the component to be blown is less than the prescribed weight for that species, seeds of that species remaining in the heavy fraction may be considered to be pure seed, but the light fraction must be searched for pure seed meeting the definitions of Section 3.2.2.c. If the Uniform Blowing Method is not used for mixture components, the definitions of Section 3.2.2.c are to be applied in determining the percentage pure seed. It is not obligatory to use the Uniform Blowing Method for any component of a mixture.

3.8 EXAMINATION OF SEEDS DIFFICULT TO IDENTIFY

3.8.1 Visually indistinguishable species when found as a contaminant

Because of the similarity in seed characteristics, kinds within each of the following groups need not be separated from one another and must be reported as designated on the label or sample. Seeds within a group must be separated from all species not in that group. Note that while overlap of seed characteristics between species within the following groups makes separation of individual seeds very difficult or impossible, bulk samples are usually identifiable. Analysts must verify that the sample is of the kind named in labelling. See also Section 3.9.8.e for methods of reporting species difficult to identify.

Visually indistinguishable species when found as a contaminant	Reported as
Allium species: Onion (Allium *cepa* L.), Leek (*Allium porrum* L.) and Chives (*Allium schoenoprasum* L.)	*Allium* sp. or spp.
Argentine Rapeseed types: Rutabaga, etc: B*rassica napus* types.	B*rassica napus*
Barley: Six-row (*Hordeum vulgare* subsp. v*ulgare)* and two-row (*Hordeum vulgare* subsp. v*ulgare*).	*Hordeum vulgare subsp. vulgare*
Bentgrass: Creeping Bentgrass (*Agrostis stolonifera* L.), Colonial Bentgrass/Browntop (*Agrostis capillaris* L.) and Redtop (*Agrostis gigantea* Roth)	*Agrostis* sp. or spp.
Brome: Sweet (*Bromus carinatus* Hook. & Arn.), California (*Bromus carinatus* var. carinatus) and Mountain (*Bromus carinatus* var. *marginatus*)	*Bromus carinatus*
Brome: Smooth (*Bromus inermis*), Meadow (*Bromus riparius* Rehmann) and Hybrid (*Bromus riparius* x *Bromus inermis*)	*Bromus* sp. or spp.
Cabbage: Cauliflower etc: *Brassica oleracea (all varieties)*.	*Brassica oleracea*
Canola: type *Brassica juncea*, Brown Mustard, Oriental Mustard, Indian Mustard: *Brassica juncea* types.	*Brassica juncea*
Chicory/endive: Chicory: Chicory Cultivated/Wild (*Cichorium intybus* L.) and Endive (*Cichorium endivia* L.)	*Cichorium* sp. or spp.
Fescues: Chewings (*Festuca rubra* subsp. *fallax**), hard (*Festuca brevipila**), red or creeping red (*Festuca rubra* subsp. *rubra**), Fine-leaved (*Festuca filiformis*), sheep (*Festuca ovina*) and various-leaved (*Festuca heterophylla*).	*Festuca* sp. or spp.
Hop Clovers: Hop or Yellow (*Trifolium aureum* Pollich), Hop or Large (*Trifolium campestre* Schreb.) and Hop or Small (Suckling) (*Trifolium dubium* Sibth.)	*Trifolium* sp. or spp.
Lespedeza: Common or Kobe (*Kummerowia striata* (Thunb.) Schindl. (=*Lespedeza striata* (Thunb.) Hook. & Arn.)), Korean (*Kummerowia stipulacea* (Maxim.) Makino (=*Lespedeza stipulacea* Maxim.)) and Sericea or Chinese (*Lespedeza cuneata* Dum.-Cours.) G. Don)	*Lespedeza* sp. or spp.
Polish rapeseed: Forage Rape, Turnip, Spinach mustard, etc: *Brassica rapa* types *(includes B. campestris, B. chinensis, B. pekinensis* and *B. perviridis*).	*Brassica spp.*
Ryegrass and Festulolium: Annual (*Lolium multiflorum*), intermediate (*Lolium x hybridum*), perennial (*Lolium perenne*) and *Festulolium* (x *Festulolium loliaceum* (Huds.) P. Fourn.)(= *Festuca pratensis* × *Lolium perenne*)	*Lolium* sp. or spp., or *xFestulolium* sp.
Sweet clover: White blossom (*Melilotus albus)* and yellow blossom (*Melilotus officinalis*).	*Melilotus* sp. or spp.
Timothy: Common timothy (*Phleum pratense*), dwarf (*Phleum bertolonii*) and Alpine (*Phleum alpinum*).	*Phleum* sp. or spp.
Wheatgrass: Fairway crested (*Agropyron cristatum*), standard crested (Agropyron *desertorum*) and Siberian (*Agropyron fragile* subsp. *sibiricum*).	*Agropyron* sp. or spp.
Wheatgrass: Intermediate (*Elytrigia intermedia* subsp. *intermedia*) and pubescent (*Elytrigia intermedia* subsp. *intermedia*).	*Elytriga intermedia* subsp. *intermedia*
Wheatgrass: Streambank (*Elymus lanceolatus* subsp. *lanceolatus*) and Northern (*Elymus lanceolatus* subsp. *lanceolatus*).	*Elymus lanceolatus subsp. lanceolatus*
Wheatgrass: Slender (*Elymus trachycaulus* (Link) Gould ex Shinners), Bearded/Awned (*Elymus trachycaulus* subsp. *subsecundus*), *Elymus trachycaulus* subsp. *trachycaulus*, and *Elymus trachycaulus* subsp. *virescens*	*Elymus trachycaulus*

*If required for information purposes, hard fescue and red or creeping red fescue can be distinguished using the ammonia fluorescence test as described in the current AOSA *Cultivar Purity Testing Handbook.* The percentage by number result from the fluorescence test cannot be converted directly to a percentage by weight because of a size difference in the seeds of the two species. Results from

a fluorescence test must be reported under remarks on the report of analysis, accompanied by the words "as determined by the ammonia fluorescence test".

3.8.2 Agropyron, Elymus, Elytrigia, Leymus, Pascopyrum, Psathyrostachys and Pseudoroegneria species

a. Single species samples of wheatgrass or wild-rye, examination for the presence of *Agropyrons Elymus, Elytrigia, Leymus, Pascopyrum, Psathyrostachys and/or Pseudoroegneria* species as contaminants, see Section 3.4.e
When the standard for non-Brassica crops is expressed by number, examine under a stereoscopic microscope the 2 gram portion of the working sample taken for pure. If "on-the-line" for either percent pure seed or percent other crop seeds, check with a further 2 grams. From the remainder of the working sample, remove all doubtful seeds, and examine them under a stereoscopic microscope. See Section 3.8.1 for groupings of species which need not be separated from each other.

b. Mixtures containing wheatgrasses or wild-rye
Depending on the percentage of *Agropyron, Elymus, Elytrigia, Leymus, Pascopyrum, Psathyrostachys and/or Pseudoroegneria* species present in the mixture separate the wheatgrass species and/or wildrye species from the quantity given in column 6, Table 1, Section 2.3.4 and if necessary any further quantity needed to make available the number of seeds of wheatgrass and/or wildrye species required for a germination test (see Section 4.4.2) for examination under a stereoscopic microscope. From the remainder of the working sample remove all doubtful seeds and examine them under a stereoscopic microscope. See Section 3.8.1 for groupings of wheatgrass species which need not be separated from each other.

c. Separation of slender wheatgrass (*Elymus trachycaulus*) from streambank wheatgrass (*Elymus lanceolatus*) and northern wheatgrass (*Elymus lanceolatus*) Slender wheatgrass, is often a common impurity in streambank or northern wheatgrass. However, since slender wheatgrass will fluoresce under ultraviolet light it is possible to separate these species. Examine the 2 gram pure seed portion of the working sample under an ultraviolet light (365 nm) in a room from which all incidental light has been excluded. Remove all seeds which fluoresce and verify their identity under a stereoscopic microscope. If considered necessary check with a further 2 grams. Since UV light may be harmful to the eyes safety goggles should be worn when making this test.

3.8.3 *Agrostis* species

a. Percentage by weight of other *Agrostis* species in a sample of *Agrostis* species (Grade Table 12)
When analysing a sample of one of the *Agrostis* species for purity, examine 200 seeds with lemmas and paleas attached. Separate and classify into (i) *Agrostis* species under analysis, and (ii) other *Agrostis* species. See Section 3.8.1 for groupings of species which need not be separated from each other. Weigh the two fractions separately and calculate percentage of each, following the example given in Section 3.8.4.b. **Note:** Any naked caryopses separated out during the selection of the 200 seeds must be added back to the "source of seed" for germination.

b. Redtop in lawn and turf mixtures: Examine to determine if redtop (*Agrostis gigantea*) is present in excess of the amount permitted under Part II, Grade Table XIV.

3.8.4 *Poa* species

a. General procedure
After the initial separation of "pure bluegrass", other crop seeds, weed seeds and inert matter have been completed using the procedure of Section 3.5, and their percentages have been calculated, extract 0.15g or at least 400 seeds (lemmas and paleas attached) from the "pure bluegrass" fraction and examine under a stereoscopic microscope, for the presence of other crop *Poa* species.

Separate the species, determine the proportion by weight of each, and from this calculate the percentage in the entire sample. Add the percentage by weight of other crop *Poa* spp. to the percentage by weight of other crop determined in the 1g analysis. See example calculation in Section 3.8.4.b.

The weedy species most likely to be encountered (e.g. *Poa bulbosa, Poa secunda*) are easily identified and must be separated from the entire 1g portion. If, however, a difficult-to-identify species is present, it should be separated from the 400-seed fraction and the percentage by weight added to the percentage by weight of weed seeds determined in the 1g analysis. **Note:** Any naked caryopses separated out during the selection of the 400 seeds must be added back to the "source of seed" for germination.

b. Method of calculation of results on a 400-seed test

Example procedure and method of calculating the results of a 400-seed separation of *Poa* spp. (from the initial 1g analysis, percentage of pure bluegrass = 89.1%, other crop seeds = 0.3%, weed seeds = 0.4%, inert = 10.2%):

	Weight	Proportion in 400 seeds	Percent of sample
372 seeds of *P. pratensis*	0.1046 g	0.1046 / 0.1097 = 95.4%	95.4% X 89.1 = 85.0%
28 seeds of *P. compressa*	0.0051 g	0.0051 / 0.1097 = 4.6%	4.6% X 89.1 = 4.1%
	0.1097 g		89.1%

Kentucky bluegrass pure seed	85.0%
Other crop seeds (0.3% + 4.1% *Poa compressa*)	4.4%
Weed seeds	0.4%
Inert	10.2%
	100.0%

3.8.5 *Brassica* and *Sinapis* species

Because of the similarity in appearance among the species of *Brassica* and *Sinapis*, all portions of the *Brassica* and *Sinapis* samples under Grade Tables VII and XIX must be examined using a stereoscopic microscope, except that for samples of *Sinapis alba*, a microscope need not be used for the third portion.

3.8.6 *Festuca pratensis* and *Festuca arundinacea*

To search for one of these species as a contaminant in the other and for the presence of *Lolium multiflorum, L. perenne* and *L. xhybridum*, either examine under a stereoscopic microscope at least 400 seeds from the "pure seed" (the kind under analysis plus seeds of the similar-appearing kinds if present), or with the aid of a magnifier make the separation on the 2 gram pure seed portion of the working sample examining microscopically only doubtful seeds. If only 400 seeds are examined, follow the procedure as described in Section 3.8.4 to calculate the percentage of pure seed and other crop seeds.

3.8.7 *Lolium* species

Ryegrass (*Lolium perenne, L. multiflorum* and *L. xhybridum*): For the presence of *Festuca pratensis* and *F. arundinacea*, make an examination following the procedures outlined in Section 3.8.6.

3.8.8 *Vicia* species except *Vicia faba* vars.

Vetches: Using magnification (e.g. stereoscopic microscope), examine 25g for species of vetch other than the one under analysis. Report the number(s) of other vetches found in this examination under "Remarks" on the report of analysis.

3.9 PURITY REPORTING PROCEDURES

All test results to be used for the grading of seed lots must be reported on a report of analysis as described in Section 1.0.

When reporting results the scientific names, common names or both may be used. However, scientific names are more universally recognized and consistent and therefore may be of greater value.

The recognized sources for:

a. Scientific names

(i). Schedule I to the *Seeds Regulations* and the *Weed Seeds Order*,

(ii). Germplasm Resource Information Network (GRIN) http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl?language=en,

(iii). ISTA *List of Stabilized Names*

(iv). *AOSA Rules for Seed Testing* Volume 3, *Uniform Classification of Weed and Crop Seeds*

(v). If not listed in any of the above, consult one of:
- Flora of North America http://floranorthamerica.org/,
- Canadian databases VASCAN http://data.canadensys.net/vascan/search/,
- Plants of Canada http://www.plantsofcanada.info.gc.ca/.

b. Common names

(i). Schedule I, to the *Seeds Regulations* and the *Weed Seeds Order*

(ii) For those species that are not listed in the Schedule I to the *Seeds Regulations* or the *Weed Seeds Order* consult:
- *Common and Scientific Names of Weeds in Canada* (current version) or *Inventory of Canadian Agricultural Weeds* http://publications.gc.ca/site/eng/243443/publication.html by Stephen Darbyshire.
- Germplasm Resource Information Network (GRIN).
- *AOSA Rules for Seed Testing* Volume 3, *Uniform Classification of Weed and Crop Seeds.*

3.9.1 Reporting results of percentage tests

a. Results obtained on tests to determine percentage by weight of components or impurities (e.g. % pure seed, % inert, % ergot, etc.) must be given to one decimal place and must total 100.0%. If the sum of the percentages does not equal 100.0% (either 99.9 or 100.1) then add or subtract 0.1% from the largest value (normally the pure seed fraction).

b. Results of less than 0.05% must be reported as trace or "TR"; 0.05% to 0.09% must be reported as 0.1%. If the percentage for a component is nil, this must be shown as "0.0" in the appropriate space. If a percentage for a component was not determined, this must be shown as "-" in the appropriate space.

3.9.2 Reporting results of tests for numbers per unit weight

a. The worksheet must show, in the appropriate spaces:

(i). the weight of each portion analysed of the working sample;

(ii). the name and actual numbers of impurities found in each portion of the working sample analysed. In the case of any prohibited noxious weed seeds found, the total number(s) are not to be included in the total of Primary plus Secondary Noxious Weed Seeds but must be included in the total of Total Weeds Seeds;

(iii). the worksheet may also show the results expressed as number per unit weight as described for the report of analysis.

b. The report of analysis must show the name and number of impurities expressed in terms of the unit weight as set out in the Grade Table (i.e. number per 25g, per 500g or per kg, depending on the Grade Table). If a larger or smaller quantity than the unit weight has actually been analysed, then the numbers of impurities must be divided or multiplied so that they are in terms of the unit weight. If by dividing, a fraction is obtained, do not round to a whole number (e.g. if 3 seeds are found in 50 grams analysed, report as 1.5 seeds per 25g, not as 2 per 25g). Consider 3.13g and 1.56g as being equal to 1/8 and 1/16 of 25g respectively, when calculating the number of foreign seeds found in 25g.

c. If the number of impurities of a category is nil, this must be reported on the worksheet and the report of analysis as "0". If the number of impurities of a category was not determined, this must be reported as "-".

d. When a reduced test for small lots of High-value seed is performed, the words “Reduced Test” and the weight of analysis must be reported under “Remarks” on the Report of Analysis.

3.9.3 Reporting of Seeds with Variable Classification

a. ***Brassica*** **species and** ***Sinapis alba***
For Grade Tables VII, VIII, IX, X, XI, and XIII, *Brassica* species and *Sinapis alba* when present as impurities must be reported separately from and not included in the total other crop seeds. For all other Grade Tables, these species must be listed with and included in the total other crop seeds.

b. **Carrot (*Daucus carota*), Radish (*Raphanus sativus*), Chicory (*Cichorium intybus*), Dandelion (Taraxacum officinale), Sorrel (Rumex acetosa) and Parsnip (Pastinaca sativa)** when found as impurities are classified as either noxious/other weed seeds or other crop seeds according to the weight of evidence as to whether they are the wild form or the cultivated form. Thus if wild radish pods are found with or without naked radish seeds, the radish must be classified as the wild form or if the radish seeds occur in a sugar beet sample from British Columbia the evidence would indicate that they are of the wild form. Where there is no evidence that will indicate whether the seed is wild or cultivated, and moreover to cover the case in which the occasional carrot, radish, chicory, dandelion, sorrel or parsnip seed inadvertently gets into other seeds in the warehouse, such seeds if found in seeds of the crop kinds listed in Grade Tables XVI through XX, or in vegetables or herbs not listed in the Grade Tables, may be classified as the cultivated form. If found in seeds of crop kinds listed in other Grade Tables they must be classified as the wild form and entered in the appropriate noxious weed section of the report of analysis.

c. **Cleavers or false cleavers (*Galium aparine* and *G. spurium*)**, when found in samples of the crops listed in Grade Table VII. List at the top of the "Secondary Noxious Weed Seeds" section, and include their number in the total secondary noxious.

d. **False wild oat (*Avena sativa* fatuoid)**, when found in samples of oat or cereal mixtures must be considered to be oat, but when found in samples of any other kind must be classified as other crop seed. Seeds of false wild oat must be examined carefully to ensure they are not wild oat (*Avena fatua and Avena sterilis*).

e. Juncus tenuis (or *Juncus* spp. having seeds of a similar size)
Individual seeds of *Juncus tenuis* or other species of *Juncus* having seeds of a similar size must be considered inert matter. Clusters or capsules of *Juncus* spp. must be left intact and included with the weed seeds. For reporting purposes, the presence but not the number of individual seeds must be reported under "Other Weeds" for Grade Table XII, XIV and XV and under "Remarks" for other Grade Tables.

f. Mayweed (*Anthemis cotula*) head
When found in the kinds or species listed in Grade Table XVI, do not disturb the seeds in the head, but report the number of heads or partial heads under "Secondary Noxious Weed Seeds" and include the number of heads in the total secondary noxious. Report the number of all loose seeds.

g. Sweet clover (*Melilotus albus* and *Melilotus officinalis*)
For Grade Tables VIII, IX, X, XI, and XIII, sweet clover must be reported separately and not included in the total other crop seeds. For all other Grade Tables, sweet clover must be listed with and included in the total other crop seeds.

h. Tartarian buckwheat (*Fagopyrum tataricum*)
When found in samples of the crops listed in Grade Tables I to III, list at the top of the "Other Crops" section, and include their number in the total other crops.

i. Wild oat (*Avena fatua* or *Avena sterilis*)
When found in samples of the crops listed in Grade Tables I to III, list at the top of the "Secondary Noxious Weed Seeds" section, and include their number in the total secondary noxious.

3.9.4 Reporting Unidentifiable free caryopses of the Poaceae

Where unidentifiable caryopses of the Poaceae are found as contaminants in a working sample, the number present must be reported as "Poaceae sp." under Other Weed Seeds on the report of analysis. For Grade Tables XII and XIV, such seeds must be included in and weighed with the "Total Weeds".

Free caryopses which are not identifiable to species, but which can be identified as belonging to the genus *Elytrigia, Avena, Lolium*, or *Sorghum* must be classified according to the appropriate noxious weed seed classification if there is evidence to support this classification, such as the presence of identifiable seeds or structures of that species. A comment must be recorded on the worksheet indicating what evidence was used to support the classification. If no such evidence is present, report the genus name under Other Crop Seeds, on the report of analysis.

3.9.5 Reporting of Agropyron, Elymus, Elytrigia, Pascopyrum and/or Pseudoroegneria species found as contaminants in non-wheatgrass samples

All contaminant seeds which are known to be slender wheatgrass (*Elymus trachycaulus*) and all seeds which cannot be distinguished by microscopic examination from slender wheatgrass, are to be reported as "slender wheatgrass". Any other seed of wheatgrass which is known not to be *Elymus repens* and cannot be identified to Genus level may be reported as "*Poaceae* sp." under Other Crop Seeds.

3.9.6 Reporting Results of More Than One Test

a. Percentage Tests

If two or more tests are carried out on the same sample, determine if the two tests are compatible by using Section 3.5.3.c Table 3. If the difference between the original and second tests exceeds the tolerance given in Table 3, conduct a third test and average the results which are compatible according to Table 3. If the third test falls between the first two and is compatible with both, then report the average of the three tests. If there are no compatible results after three check tests, analyse another working sample (not more than four tests in total). Report the weighted average of the check tests for which the highest and lowest results do not differ by more than twice the tolerance stated in Table 3, unless it is apparent that one or more of these results are due to an error and not to random sample variation. In that case, discard the test(s) with the errors. If no pair of results is within tolerance, it is advisable to find the cause of the variation.

b. Tests for numbers of impurities per unit weight

If two or more tests are carried out on the same sample, or if a larger quantity than the minimum specified in Section 2.3 is analysed, then the total number of impurities in the total quantity analysed must be used in deriving the reported number of impurities per unit weight.

3.9.7 Reporting of Varietal Contaminants

When, during the course of the purity analysis, seeds are observed which appear to be of a variety other than the one under analysis, this observation must be reported as accurately as possible. This is not interpreted to mean that the purity analysis is a purity of variety test. Suspected varietal contaminants are to be noted on the report of analysis under "Remarks", as in the following example.

"Observed 5 yellow seeds in 500g analysed."

3.9.8 Additional Reporting Requirements

a. Crop Kinds Not Listed in the Seeds Regulations

A sample of a species not listed in the Seeds Regulations must be analysed and reported as a crop kind (see Section 3.4.1); but when such seeds are found as an impurity in seed of any other crop they must be classified and reported as weed seeds.

b. Noxious Weed Seeds

(i). All seed lots must be free from prohibited noxious weed seeds listed in the Weed Seeds Order (*Seeds Regulations* paragraph 7(1)(a)). Note: Certain species may have a variable classification, as outlined in Section 3.9.3.

1. A Canadian Accredited laboratory, must notify Seed Section of the finding of prohibited noxious weed seeds at cfia.seed-semence.acia@canada.ca
   - If the prohibited noxious weed seeds is found in any seed sample during the analysis of the working sample(s)
   - If the prohibited noxious weed seeds is observed in the unanalyzed portion. A statement must be made on the final report of analysis indicating that the lot must not be graded due to the presence of a prohibited noxious weed seed(s). This does not imply that the analyst must examine the unanalyzed portions of the sample.
2. Include the name of the species identified, the crop kind in which the prohibited noxious weed seed was found and the country of production of the seed lot.

(ii). If Noxious Weed Seeds are observed in an Unanalyzed Portion of the sample this information must be recorded in the "Remarks" section of the worksheet. For non-prohibited species, this information is not considered for grading decisions. For prohibited noxious species see Section 3.9.8.b.i.

(iii). The presence of inert seed structures from plants classified as noxious weeds in the *Weed Seeds Order* found during the analysis of the working sample(s) must be recorded under "Remarks" on the worksheet as information in case of future enquiries. This information need not be reported on the final report of analysis issued by the laboratory.

c. Multiple seed structures found as contaminants

When found as contaminants, multiple seed structures such as pods, heads, capsules, spikelets, etc., must be broken into individual seeds, each being counted and reported as one seed. This does not always apply to *Juncus* (see Section 3.9.3.f) or heads of Mayweed (see Section 3.9.3.g).

d. Procedure for Samples Too Small For Complete Analysis

When there is insufficient seed for a complete purity analysis, report the results on the report of analysis, and under "Remarks" include the statement: "Insufficient seed for complete analysis — do not grade"

e. Reporting of species difficult to identify

When it is not possible to positively identify seed to the species level, report the genus name followed by 'sp', as in *Lolium* sp. If it is possible to distinguish it from seeds of another group within the genus, the analyst may use 'sp.' followed in parentheses by 'cf.' and the name of the species which it most closely resembles, as in *Festuca* sp. (cf. *rubra*). Multiple species names may be used where there is greater uncertainty, as in *Festuca* sp. (cf. *rubra/brevipila*). Where common names are used, report as "Fescue (resembles red or hard)". When it is not possible to identify a seed to the genus level, report the family name followed by 'sp', as in Poaceae sp. Where species are listed as Prohibited noxious with the same Genus but different species (i.e. Bothriochloa ischaemum, Bothriochloa laguroides) the analyst must identify them to species level. If the analyst is unable to identify the seed to the species level, they must submit the seed to SSTS for identification.

3.10 PURITY PROCEDURES FOR COATED SEED

NOTE: Standards and labelling requirements for coated seed are outlined in Sections 9 and 31 of the *Seeds Regulations*. The grade of the seed or seed mixture must be established prior to coating. In addition, the percentage by weight of seed must appear on the label. Because of the nature of the coated seed product and the procedures for testing, it is not valid to compare percentage by weight values determined by testing de-coated seed, with the standards given in the Grade Tables. Procedures for determining percentages by weight of coated seed are therefore not given here. If necessary for information purposes, the procedures for testing coated seed given in the current "Rules for Testing Seeds", published by the Association of Official Seed Analysts, must be used. The following procedure may be used to determine if imported seed meets the minimum standards for foreign seeds.

3.10.1 Definition

Coated seed (includes pelleted and encrusted seed units) is a seed unit covered with any substance which changes the size, shape, or weight of the original seed. Seeds coated with ingredients such as, but not limited to, rhizobia, dyes, and pesticides are excluded.

3.10.2 Obtaining the working sample

The minimum submitted sample size for samples of coated units to be submitted for a foreign seed determination must be approximately 50,000 units. Due to variation in weight of coating materials, the size or weight of the working sample must be determined separately for each lot. This weight must be determined by weighing 100 completely coated units and calculating the weight of 25,000 units for the foreign seed determination. Use the methods described in Section 2.2 to obtain the working sample.

3.10.3 Foreign seed determination

To determine the number of foreign seeds (i.e. noxious weeds, other weeds and other crops) per unit weight, examine approximately 25,000 units which have been de-coated. Remove the coating material from the seed by washing with water or other solvents such as, but not limited to, vinegar (5%

acetic acid) or dilute sodium hydroxide. Use of fine mesh sieves is recommended for this procedure and stirring or shaking the coated units may be necessary to obtain de-coated seed. Spread on blotters or filter paper in a shallow container. Air dry overnight at room temperature.

4.0 GERMINATION

4.1 INTRODUCTION

The object of testing for germination is to determine the maximum germination potential of the seed. Testing under field conditions is normally unsatisfactory, as the results cannot be repeated with reliability. Laboratory methods have, therefore, been evolved in which the external conditions are controlled to give the most regular, rapid and complete germination for the majority of samples of a particular species. The conditions have been standardized to enable the test results to be reproduced within limits as near as possible to those determined by random sample variation. This chapter provides the methods and procedures to be used for germination testing for the purpose of grading seed.

4.2 METHODS TO BE USED

4.2.1 Prescribed Methods

The laboratory methods prescribed in Section 4.6.2 (Table 5) must be used when germination tests are to be used as the basis for grading seed under the Seeds Regulations, except that modified methods may be used according to Section 4.2.2.

4.2.2 Modified Methods

On those exceptional occasions when samples do not respond to the prescribed methods to give the germination potential, an appropriate modified method may be used as follows:

a. The analyst must be certain that the prescribed methods of Table 5 do not produce results which truly reflect the maximum germination potential of the sample or samples under test.

b. The modified method must enable the analyst to report germination as defined in Section 4.3.1, i.e. the condition of essential seedling structures must be assessed.

c. The analyst must have reasonable grounds to expect that the method to be used is appropriate for the specific problem at hand and gives reproducible results. Published methods must be used wherever possible.

d. The analyst must be competent in the use of the method.

e. The method used must be clearly indicated on the report of analysis.

4.3 DEFINITIONS

4.3.1 Seed Germination

In seed laboratory practice, germination is defined as the emergence and development from the seed embryo of those essential structures which, for the kind of seed under test, are indicative of the ability to produce a useful, mature plant under favourable field conditions. In a laboratory germination test, the plant-producing potential of a sample of seed is evaluated.

4.3.2 Normal Seedlings

Seedlings possessing the essential structures that are indicative of their ability to produce useful mature plants under favourable field conditions. Detailed seedling descriptions are given in Section 4.14.

4.3.3 Abnormal Seedlings

All seedlings which cannot be classified as normal seedlings. Detailed descriptions of abnormal seedlings are given in Section 4.14. All seedlings exhibiting negative geotropism are to be classified as abnormal.

4.3.4 Ungerminated Seeds

a. Fresh Seeds
Seeds which have failed to germinate but have imbibed moisture and appear firm, fresh, and capable of germination at the end of the prescribed test period and under the prescribed test conditions. Such seeds may be viable but dormant.

b. Dormant Seeds
Viable seeds, other than hard seeds, which fail to germinate when, provided the prescribed germination conditions.

c. Hard Seeds
Seeds which remain hard at the end of the prescribed test period because their impermeable seed coats prevent the absorption of water. See Section 4.10.7.

d. Dead Seeds
Seeds which at the end of the test period are neither hard nor dormant nor have produced any part of a seedling.

4.4 PREPARATION OF SEEDS FOR GERMINATION TESTS

4.4.1 Source of Seeds for Germination

a. From the pure seed separation: For germination of grasses in Grade Tables XI, XII, XIV and XV, seeds must be taken, without discrimination, from the pure seed separated in the purity test.

b. From the submitted sample: For germination of all kinds other than those mentioned in Section 4.4.1.a, seeds must be taken directly from the submitted sample after careful mixing as described in Section 2.2. The seeds for germination must be taken without discrimination except that they must be "pure seed" as defined in Section 3.2.2. The following seed structures are considered as seed units and need not be separated for germination: multiple florets in oats, entire spikelets in emmer and spelt, and entire schizocarps of the Apiaceae.

4.4.2 Number of Seeds for Germination

a. **Individual kinds of seeds:** At least 200 pure seeds must be tested for germination. Four hundred seeds of any kind may be planted if considered desirable. The seeds must be tested in replicates of 100, 50, 25, or 10 seeds as appropriate.

b. **Mixtures of forage seeds, Grade Table XIII, lawn or turf mixtures, Grade Table XIV or ground cover mixtures, Grade Table XV**: Plant at least 100 pure seeds of each kind comprising 15% or less of the mixture and 200 seeds of each kind in excess of 15%. Four hundred seeds of any kind may be planted if considered desirable. Components comprising less than 5% of the mixture must be planted only if they are to be named on the label as a component. Report the percentage germination of each kind separately. For germination purposes, the following groupings may be made:

***Agrostis*:** When two or more *Agrostis* species are present in a mixture, they need not be separated and may be germinated together as one component of the mixture.

Festuca: For germination purposes, the *Festuca* species may be considered as belonging to three groups. Seeds of each group must be separated from seeds of the other groups, but seeds of the kinds within a group need not be separated from one another:

(i). Meadow fescue

(ii). Tall fescue

(iii). Chewings, hard and red or creeping red fescue, fine-leaved, hard, sheep and various-leaved fescue.

c. **Mixtures of cereal seeds, Grade Table III**: Plant at least 100 pure seeds of each kind comprising 50% or less of the mixture and 200 seeds of any kind comprising more than 50% of the mixture. Components comprising less than 5% of the mixture must be planted only if they are to be named on the label as a component. Report the percentage germination of each kind separately

4.5 GERMINATION CONDITIONS

4.5.1 Planting of Seeds

Seeds should be adequately and uniformly spaced so that contact of adjacent seeds is avoided. Counting and planting may be done by hand, ensuring seed selection is random and in accordance with the pure seed definitions, or with planting aids provided these do not introduce a bias when the seed is selected. If a vacuum counter is used, the head must be held flat and completely covered with seed before the vacuum is turned on to avoid biased selection of seed.

4.5.2 Substrata and Moisture

All substrata, containers and moistening agents must be non-phytotoxic. New shipments of substrata must be tested for phytotoxicity according to Section 4.5.6.

The substrata must have a pH value within the range 6.0 - 7.5 when moistened. The substratum must be moist enough to supply the needed moisture to the seeds at all times. Avoid supplying excessive moisture which will restrict aeration of the seeds. Except as provided for those kinds requiring high moisture levels, the substratum should never be so wet that a film of water is formed around the seeds. For most kinds of seeds, blotters or other paper substrata must not be so wet that by pressing, a film of water forms around the finger.

The initial quantity of water to be added will depend on the nature and dimensions of the substratum and also on the size and species of the seed to be tested. The optimum amount should be determined by experiment.

Subsequent watering should be avoided wherever possible as it is likely to increase variability between replicates and between tests. Since the rate of evaporation will depend on the relative humidity of the air, it is desirable to keep water in the germination chambers or to provide other means of supplying a relative humidity of approximately 95 percent. Germination tests must be inspected at suitable intervals to ensure that an adequate moisture supply is available at all times.

a. Sand. Sand for germination tests should be practically free of organic matter, soluble salts and clay or fine silt. At least 90% of the particles must pass through a sieve with holes or meshes of 2.0 mm width. To improve the water holding capacity of the sand, vermiculite may be added. Sand is used for the following methods:

(i). **S (In sand).** Seeds are placed on a level layer of moist sand and covered with a layer of uncompressed sand to a depth of 10 to 20 mm, depending on the size of the seed. The quantity of water added will depend on the particle size of the sand, the characteristics of the vermiculite when added, and the characteristics of the seeds to be planted. The optimum

amount of water to add should be determined by experiment. As a guideline, for 24-mesh sand with no vermiculite added, add 125 mL water per litre of sand, or add water until the sand can be formed into a ball when squeezed in the palm of the hand, the ball breaking freely when pressed between two fingers.

(ii). **TS (Top of sand).** Seeds are pressed into the surface of the sand. No covering layer is added. Moisture levels are defined as follows:

Standard: Two or three drops of water show when container is tipped to 45° angle.
Light: No free water shows when container is tipped.
Heavy: Small pool of water shows when container is tipped.

b. **Paper.** Blotters, towels, filter paper and cellucotton must be wood, cotton or other purified vegetable cellulose. The paper should be such that the roots of the seedlings will grow on and not into it and it possesses sufficient strength to enable it to resist tearing when handled during the test. For paper tests moisture levels are defined as follows:

Standard: Soak until saturated. Drain until begins to drip.
Light: Soak until saturated. Drain thoroughly and press against a dry absorbent surface to remove excess moisture.
Heavy: Soak until saturated. Do not drain.

Paper is used in the following methods:

(i). **BP (Between paper).** Papers of an appropriate size for the crop kind under analysis are used, the seeds being spaced on one half of the moist paper before the other half is folded over (e.g. for wheat, papers measuring 16x28 cm folded to 16x14 cm would be appropriate).

(ii). RP (Raised paper). The paper are prepared as for BP, but to improve aeration of the seeds, the top paper is raised from the seed by means of corks, vial tops, narrow strips of blotter, etc.

(iii). **TP (Top of paper).** Seeds are placed on top of standard germination paper or filter paper.

(iv). RT (Rolled paper (towels)). Seeds are evenly spaced on two sheets of standard weight (38 lb) or a single sheet of heavy weight (76 lb) germination paper, then covered with a single sheet. The paper is rolled and placed in an upright position. The paper towelling should be moistened until its wet weight is about three times that of its dry weight.

(v). PP (Pleated paper). The seeds are placed in a pleated, accordion-like filter paper strip with 50 pleats, usually two to a pleat. This method may be used as an alternative where any paper substratum is prescribed.

c. Soil-less organic growing media (O). Samples may be retested in a soil-less organic growing media to confirm tests made by other methods, for example testing samples which produced seedlings showing symptoms of phytotoxicity when germinated on paper or in sand. The soil-less mix should be a good quality, organic soil-less potting mix. The soil-less mix must have a pH value within the range 6.0 – 7.5. Water should be added until the soil-less mix can be formed into a ball when squeezed in the palm of the hand, the ball breaking freely when pressed between two fingers.

4.5.3 Water

The water used to moisten the substrata must be reasonably free from organic or inorganic impurities. If the usual water supply is not satisfactory, distilled or de-ionized water may be used.

4.5.4 Temperature

Temperatures are prescribed in Section 4.6.2 Table 5 and must be determined at the level of the seeds on the substratum. The temperature must be as uniform as possible throughout the germination or prechill chamber. Care must be taken that heat generated by the light source does not cause the temperature to exceed the temperature level prescribed. The temperature indicated must be regarded as a maximum and variation due to the apparatus must not be more than ± 2°C.

Single numerals in the temperature column of Table 5 indicate that the seed must be germinated at a constant temperature. Two numerals separated by a dash indicate that the seed must be germinated with an alternation of temperatures, the test being held at the lower temperature for approximately 16 hours per day, and at the higher temperature for 8 hours per day. When testing seeds that are dormant, it is essential that the change-over of temperature be accomplished in one hour or less. If the tests are not given alternating temperatures over weekends and on public holidays, they may be held at the lower temperatures during such times.

When prechilling (rechilling or midchilling) is required for breaking dormancy, a constant temperature of not less than 5°C or more than 10°C must be selected, maintained and used as described in Section 4.8.1.

4.5.5 Light

Seeds of most of the species in Section 4.6.2 Table 5 will germinate either in light or in darkness. However, illumination is generally recommended, as better developed seedlings are produced which are more easily evaluated. If light is known to be necessary or beneficial to induce complete germination of any given kind of seed, its use is indicated in Table 5 in the column headed "Additional Directions".

Light must be provided by a cool white fluorescent source for 8 hours in every 24. Where the seeds are germinated at alternating temperatures, they must be illuminated during the high temperature period. Seeds for which light is prescribed in Section 4.6.2 Table 5 must be germinated on top of the substratum, at an intensity of 750-1200 lux. Iluminance of seed for which light is not prescribed may be as low as 250 lux.

4.5.6 Phytotoxicity Testing

A phytotoxicity test must be conducted to compare a substratum of unknown quality with one in stock of acceptable quality. For this test, seeds of certain species which are known to be sensitive to toxic substances in the substratum are used: *Agrostis gigantea, Allium cepa, Apium graveolens, Cichorium intybus, Hordeum vulgare, Festuca rubra, Lepidium sativum* and *Phleum pratense*. At least two species must be included in the test. The seed is to be of a known quality with high germination.

The evaluation is made by comparing the root development of the seedlings of the two species grown on the two sources of substratum. The evaluation is to be made on or before the days specified in Section 4.6.2 Table 5 for first count of the species used for the test, because symptoms due to toxic substances are more pronounced at an early stage of root growth. Such symptoms are shortened roots and sometimes discoloured root tips, roots raised from the substratum, and root hairs 'bunched'. In grasses, coleoptiles may be flattened and shortened.

4.6 GERMINATION METHODS

4.6.1 Outline of Germination Methods

The prescribed methods as given in Section 4.6.2 Table 5 must be used. When alternate methods are indicated, one of them (any combination of substratum and temperature) may be used. If a sample does not respond satisfactorily to the method selected, it may be retested by one or more of the alternate methods or by a modified method as described in Section 4.2.2. The sequence of alternate methods in Table 5 does not indicate any preference.

Methods in the "General requirements" column of Table 5 must be used. Methods in the "Fresh or dormant seeds" column may be used when dormant seed is known or suspected to be present. These are described in Section 4.8 and may be applied to the original test, or to retests.

When a kind of seed for which a method is not given in Table 5 is received for germination, the current ISTA or AOSA Rules for testing seeds are to be consulted for an appropriate method. For kinds for which there is no published method, the method for a closely related species should be followed. The method used must be clearly indicated on the report of analysis.

The abbreviations used in Table 5 are defined as follows:

BP	-	Between paper (See Sec. 4.5.2.b.1)
RP	-	Raised paper (See Sec. 4.5.2.b.2)
TP	-	Top of paper (See Sec. 4.5.2.b.3)
RT	-	Rolled paper e.g towels (See Sec. 4.5.2.b.4)
PP	-	Pleated paper (See Sec. 4.5.2.b.5)
S	-	In sand (See Sec. 4.5.2.a.1)
TS	-	Top of sand (See Sec. 4.5.2.a.2)
KNO_3	-	Use solution of potassium nitrate instead of water. (See Sec. 4.8.2)
GA_3	-	Use solution of gibberellic acid instead of water. (See Sec. 4.8.4)
TZ	-	Tetrazolium (See Sec. 4.7.6)

Other abbreviation:

O	-	Soil-less organic growing media (See Sec. 4.5.2.c)

4.6.2 Table 5. Germination Methods

Kind of Seed	Substrata	Temperature (°C)	First Count (days)	Final Count (days)	Additional Directions	
					General Requirements	Fresh or Dormant Seeds
Abelmoschus esculentus Okra	S	20-30	7	14		
Agropyron cristatum Wheatgrass, crested	TP	15-25	7	14	Light	Prechill; KNO_3
Agropyron desertorum Wheatgrass, crested	TP	15-25	7	14	Light	Prechill; KNO_3
Agropyron fragile subsp. *sibiricum* Wheatgrass, Siberian	TP	15-25	7	14	Light	Prechill; KNO_3
Alternate method for *Agropyron*, spp.	TP	10-30	7	14	Light	Prechill; KNO_3
Agrostis canina Bentgrass, velvet	TP	15-25	10	21	Light *; KNO_3	Prechill
Agrostis capillaries Bentgrass, colonial, (browntop)	TP	15-25	10	21	Light	Prechill; KNO_3
Agrostis gigantea Redtop	TP	15-25	7	10	Light	Prechill; KNO_3
Agrostis stolonifera Bentgrass, creeping	TP	15-25	7	21	Light	Prechill; KNO_3
Allium cepa Onion	BP; S; RT	20	7	14	Lightly covered (sand test)	
Allium porrum Leek	BP; S; RT	20	7	14	Lightly covered (sand test)	
Allium schoenoprasum Chives	BP; S; RT	20	7	14	Light moisture; Lightly covered (sand test)	
Alopecurus arundinaceus Foxtail, creeping	TP	15-30	7	21	Light; KNO_3	
Alopecurus pratensis Foxtail, meadow	TP	15-25	7	14	Light	Prechill
Anethum graveolens Dill	BP; TS; RT	20-30	7	21		
Anthriscus cerefolium Chervil	TS; BP; RT	20-30	7	21	Light	Prechill
Anthyllis vulneraria Vetch, kidney	BP; RT	20	5	10		
Apium graveolens var. *dulce* Celery	TP	15-25; 20	10	21	Light; water only	Prechill
Apium graveolens var. *rapaceum* Celeriac	TP	15-25; 20	10	21	Light; water only	Prechill
Arrhenatherum elatius Oatgrass, tall	TP	15-25	7	14	Light	Prechill
Asparagus officinalis Asparagus	S; BP; RT	20-30	7	21	Presoak 24 hours	
Astragalus cicer Milkvetch, cicer	BP; RT	20; 15-25	7	21		
Avena nuda Oat, hulless	S; BP; RT	20		7		Prechill; Preheat
Avena sativa Oat	S; BP; RT	20		7		Prechill; Preheat

Kind of Seed	Substrata	Temperature (°C)	First Count (days)	Final Count (days)	Additional Directions	
					General Requirements	Fresh or Dormant Seeds
Beta vulgaris subsp. *vulgaris* Beet	RP; BP; PP	20; 20-30	3	10	See Section 4.7.2 and 4.10.6.a	Prechill
Beta vulgaris subsp. *vulgaris* Beet, sugar	RP; BP; RT; PP	20	3	10	See Section 4.7.2 and 4.10.6.a	
Beta vulgaris subsp. *vulgaris* Mangel	RP; BP; PP	20; 20-30	3	10	See Section 4.7.2 and 4.10.6.a	Prechill
Beta vulgaris subsp. *vulgaris* Swiss chard	RP; BP; PP	20; 20-30	3	10	See Section 4.7.2 and 4.10.6.a	Prechill
Brassica juncea Mustard, brown	TP	15-25; 25	4	7	Light	Prechill
Brassica juncea Mustard, greens	TP	15-25; 25	4	10	Light	Prechill
Brassica juncea Mustard, Indian	TP	15-25; 25	4	7	Light	Prechill
Brassica juncea Mustard, oriental	TP	15-25; 25	4	7	Light	Prechill
Brassica juncea Rapeseed,oilseed rape, canola – *Brassica juncea* type	TP	15-25; 25	4	7	Light	Prechill
Brassica napus var. napobrassica Rutabaga (swede)	TP	15-25; 25	4	10	Light	Prechill
Brassica napus var. *napus* Rape, forage	TP	15-25; 25	4	10	Light	Prechill
Brassica napus var. *napus* Rapeseed, oilseed rape, canola - Agentine type	TP	15-25; 25	4	7	Light	Prechill
Brassica nigra Mustard, black	TP	15-25; 25	4	7	Light	Prechill
Brassica oleracea var. *botrytis* Cauliflower	TP	15-25; 25	4	10	Light	Prechill
Brassica oleracea var. *capitata* Cabbage	TP	15-25; 25	4	10	Light	Prechill
Brassica oleracea var. *gemmifera* Brussel sprouts	TP	15-25; 25	4	10	Light	Prechill
Brassica oleracea var. *gongylodes* Kohlrabi	TP	15-25; 25	4	10	Light	Prechill
Brassica oleracea var. *italica* Broccoli	TP	15-25; 25	4	10	Light	Prechill
Brassica oleracea var. *sabellica* Kale	TP	15-25; 25	4	10	Light	Prechill
Brassica oleracea var. *viridis* Collards	TP	15-25; 25	4	10	Light	Prechill

Kind of Seed	Substrata	Temperature (°C)	First Count (days)	Final Count (days)	Additional Directions	
					General Requirements	Fresh or Dormant Seeds
Brassica oleracea var. *viridis* Kale	TP	15-25; 25	4	10	Light	Prechill
Brassica rapa subsp. *campestris* Rape, forage	TP	15-25; 25	4	10	Light	Prechill
Brassica rapa subsp. *campestris* Rapeseed, oilseed rape, canola - Polish type	TP	15-25; 25	4	7	Light	Prechill
Brassica rapa subsp. *chinensis* Cabbage, Chinese	TP	15-25; 25	4	10	Light	Prechill
Brassica rapa subsp. *pekinensis* Cabbage, Chinese	TP	15-25; 25	4	10	Light	Prechill
Brassica rapa var. *perviridis* Spinach mustard	TP	15-25; 25	4	10	Light	Prechill
Brassica rapa subsp. *rapa* Turnip	TP	15-25; 25	4	10	Light	Prechill
Bromus carinatus Bromegrass, sweet	TP	15-25	7	14		Prechill; KNO_3
Bromus inermis Bromegrass smooth	TP	15-25	7	14		Prechill
Bromus riparius Bromegrass, meadow	TP	20-30; 15-25	7	14		Prechill
Campanula rapunculus Rampion	TP	15; 20	7	14		
Cannabis sativa subsp. s*ativa* Hemp	BP; RT	20-30	3	7		
Capsicum annuum Pepper	RP; TP; BP; RT	20-30	7	14	Light moisture	Light; KNO_3
Carthamus tinctorius Safflower	BP; S; RT	20; 15	5	14	See Section 4.14.3 Note 7	
Cicer arietinum Chickpea	S	20-30; 25	4	7		
Cichorium endivia Endive	TS; TP	20-30	4	10	Light; heavy moisture at first See Section 4.14.3 Notes 7 and 8	Soak 24 hours in H_2O; Prechill; KNO_3
Cichorium intybus Chicory, cultivated	TS; TP	20-30	4	10	Light; heavy moisture at first See Section 4.14.3 Note 7	Soak 24 hours in H_2O; KNO_3; Prechill
Citrullus lanatus var. *citroides* Citron	BP; S; RT	25; 20-30	4	10	Light Moisture See Section 4.14.7 Note 3	May retest at 30°C
Citrullus lanatus var. *lanatus* Watermelon	BP; S; RT	25; 20-30	4	14	Light moisture See Section 4.14.7 Note 3	May retest at 30°C
Cucumis anguria Gherkin	BP; S; RT	25; 20-30	4	8	Light moisture See Section 4.14.7 Note 3	

Kind of Seed	Substrata	Temperature (°C)	First Count (days)	Final Count (days)	Additional Directions	
					General Requirements	Fresh or Dormant Seeds
Cucumis melo subsp. *melo* Cantaloupe, melon	BP; S; RT	25; 20-30	4	10	Light moisture See Section 4.14.7 Note 3	
Cucumis sativus Cucumber	BP; TP; S; RT	25; 20-30	4	7	Light moisture See Section 4.14.7 Note 3	
Cucurbita spp. Pumpkin, squash (summer or winter)	BP; S; RT	25; 20-30	4	7	Light moisture See Section 4.14.7 Note 3	
Cynara cardunculus Artichoke	BP; RT	20-30	5	14	Lightly covered See Section 4.14.3 Note 7	
Cynara cardunculus Cardoon	S; BP; RT	20-30	5	14	See Section 4.14.3 Note 7	
Cynosurus cristatus Crested dogtail	TP	20-30	7	21	Light	Prechill
Dactylis glomerata Orchardgrass	TP	15-25	7	14	Light	Prechill; KNO_3
Daucus carota subsp. *sativus* Carrot	BP; RT	20-30	5	14		
Echinochloa esculenta Millet, Japanese	BP; S; RT	20-30; 25	4	7		Sharp alternations of temperature
Echinochloa frumentacea Millet, Japanese	BP; S; RT	20-30; 25	4	7		Sharp alternations of temperature
Elymus dahuricus Wildrye, Dahurian	TP	15-25	7	14	Light	Prechill
Elymus lanceolatus subsp. *lanceolatus* Wheatgrass, northern	TP	15-25	7	21		Prechill; KNO_3
Elymus lanceolatus subsp. lanceolatus Wheatgrass, streambank	TP	15-25	7	14	Light	Prechill; KNO_3
Elymus trachycaulus Wheatgrass, slender	TP	15-25	7	14	Light	Prechill; KNO_3
Alternate method for *Elymus* spp.	TP	10-30	7	14	Light	Prechill; KNO_3
Elytrigia elongata Wheatgrass, tall	TP	15-25	5	21	Light	Prechill; KNO_3
Elytrigia intermedia subsp. intermedia Wheatgrass, intermediate	TP	15-25	5	21	Light	Prechill; KNO_3
Elytrigia intermedia subsp. *intermedia* Wheatgrass, pubescent	TP	15-25	5	21	Light	Prechill; KNO_3
Alternate method for *Elytrigia* spp.	TP	10-30	7	14	Light	Prechill; KNO_3
Fagopyrum esculentum Buckwheat, common	BP; RT	20-30	4	7		
Fagopyrum tataricum Buckwheat, tartarian	BP; RT	20-30	4	7		
Festuca arundinacea Fescue, tall	TP	15-25	7	14	Light	Prechill

Kind of Seed	Substrata	Temperature (°C)	First Count (days)	Final Count (days)	Additional Directions	
					General Requirements	Fresh or Dormant Seeds
Festuca brevipila Fescue, hard	TP	15-25	7	21	Light	Prechill
Festuca filiformis Fescue, fine-leaved	TP	15-25	7	21	Light	Prechill
Festuca heterophylla Fescue, various-leaved	TP	15-25	7	21	Light	Prechill
Festuca ovina Fescue, sheep	TP	15-25	7	21	Light	Prechill
Festuca pratensis Fescue, meadow	TP	15-25	7	14	Light	Prechill
Festuca rubra subsp. *fallax* Fescue, Chewing's	TP	15-25	7	21	Light	Prechill
Festuca rubra subsp. *rubra* Fescue, red and creeping red	TP	15-25	7	21	Light	Prechill
Glycine max Soybean	S; RT	25		7	See Section 4.7.7 and M&P Addendum Soybean Seedling Evaluation	
Helianthus annuus Sunflower	S; BP; RT	20-30; 20; 25	4	7	Light moisture See Section 4.14.3 Note 7	Prechill
Helianthus annuus Sunflower (hybrid)	S; BP; RT	20-30; 20; 25	4	7	Light moisture See Section 4.14.3 Note 7	Prechill
Helianthus annuus Sunflower (open pollinated)	S; BP; RT	20-30; 20; 25	4	7	Light moisture See Section 4.14.3 Note 7	Prechill
Hordeum vulgare subsp. *vulgare* Barley (six-row, two-row, hulless)	S; BP; RT	20		7		Prechill, Preheat
Kummerowia stipulacea Lespedeza, Korean	BP; RT	20-35; 20	5	14		
Kummerowia striata Lespedeza, Common or kobe	BP; RT	20-35; 20	7	14		
Lactuca sativa var. *angustana* Celtuce/Celtus	TP	20; 15		7	Light See Section 4.14.2 Notes 5 and 6	Prechill
Lactuca sativa var. *capitata* Lettuce - head	TP	20; 15		7	Light See Section 4.14.2 Notes 5 and 6	Prechill
Lactuca sativa var. crispa Lettuce - leaf	TP	20; 15		7	Light See Section 4.14.2 Notes 5 and 6	Prechill
Lactuca sativa var. *longifolia* Lettuce - cos (romaine)	TP	20; 15		7	Light See Section 4.14.2 Notes 5 and 6	Prechill

Kind of Seed	Substrata	Temperature (°C)	First Count (days)	Final Count (days)	Additional Directions	
					General Requirements	Fresh or Dormant Seeds
Lens culinaris Lentil	BP; S; RT	20	7	10		
Lepidium sativum Cress, garden	BP; RT	20	4	10		Prechill
Lespedeza cuneata Lespedeza, sericea or chinese	BP; RT	20-35; 20	7	28		
Leymus angustus Wildrye, Altai	TP	15-25	7	14	Light	Prechill
Linum usitatissimum Flax, oilseed and fibre	BP; RT	20	4	7	Light moisture	Prechill
Alternate method for *Linum* sp	BP; RT	15	4	10	Light moisture	Prechill
Lolium multiflorum Ryegrass, annual	TP	15-25	7	14	Light	Prechill; KNO_3
Lolium perenne Ryegrass, perennial	TP	15-25	7	14	Light	Prechill; KNO_3
Lolium x *hybridum* Ryegrass, intermediate	TP	15-25	7	14	Light	Prechill; KNO_3
Lotus corniculatus Trefoil, bird's-foot	BP; RT	20; 15	5	12		
Lupinus spp. Lupine, lupin (grain and forage)	S; BP; RT	20	5	10		
Lycopersicon esculentum Tomato	BP; TP; RP; RT	20-30	4	14		Light; KNO_3
Medicago lupulina Medick, black	BP; RT	20		5		
Medicago sativa Alfalfa	BP; RT	20		5		
Alternate method for *Medicago* spp.	BP; RT	15		7		
Melilotus albus Clover, sweet - white blossom	BP; RT	20; 18		5	Plant *Melilotus* spp. separately from other tests.	
Melilotus officinalis Clover, sweet - yellow blossom	BP; RT	20; 18		5	Plant *Melilotus* spp. separately from other tests.	
Alternate method for *Melilotus* spp.	BP; RT	15		7	Plant *Melilotus* spp. separately from other tests.	
Nasturtium officinale Watercress	TP; BP; Cellucotton; Filter paper	20-30	4	14	Light; on saturated absorbent cotton (or 4 layers saturated filter paper) in covered petri dishes.	
Nicotiana tabacum Tobacco, flue-cured and burley types	TP	20-30; 30	7	14	KNO_3	
Onobrychis viciifolia Sainfoin	BP; S; RT	20-30; 20; 15	7	14		
Panicum miliaceum subsp. *miliaceum* Millet, proso	BP; S; RT	20-30; 25	4	7		Sharp alternations of temperature

Kind of Seed	Substrata	Temperature (°C)	First Count (days)	Final Count (days)	Additional Directions	
					General Requirements	Fresh or Dormant Seeds
Pascopyrum smithii Wheatgrass, western	TP	15-25	7	28	Dark	Do not prechill; KNO_3; TZ on remaining seeds; See Section 4.7.1
Alternate method for *Pascopyrum smithii* Wheatgrass, western	TP	10-30	7	14	Light	Do not prechill; KNO_3; TZ on remaining seeds; See Section 4.7.1
Pastinaca sativa subsp. *sativa* Parsnip	BP; TS; RT	20-30	7	28	Light moisture	Prechill; may retest at 10-30°C
Alternate method for *Pastinaca sativa* subsp. *sativa* Parsnip	S	20-30; 15-25	10	28	Standard moisture	Plant on top of sand, lightly cover seeds with sand, cover test with wet blotters. May prechill 4-5 days; may retest at 10-30°C.
Pennisetum glaucum Millet, pearl	BP; S; RT	20-30; 25	4	7		Sharp alternations of temperature
Petroselinum crispum Parsley	TS; TP	15-25	10	28		May retest at 10-30°C
Phalaris arundinacea Canarygrass, reed	TS; TP	15-25; 20-30	7	21		Light; Prechill; KNO_3
Phalaris canariensis Canarygrass	BP; RT	15-25	7	10		Prechill; 0.1% KNO_3
Phaseolus coccineus Bean, runner	S; RT	20; 25	7	10		
Phaseolus lunatus Bean, lima	S; RT	20; 25	7	10	Heavy moisture first few days.	
Phaseolus vulgaris Bean, field	S; RT	25		7		
Phaseolus vulgaris Bean, garden	S; RT	25		7		See Section 4.7.3
Phleum bertolonii Timothy - dwarf	TP	15-25	7	10		Prechill
Phleum pratense Timothy - common	TP	15-25	7	10		Prechill
Pisum sativum Pea, field	S; RT	20		7		
Pisum sativum Pea, garden	S; RT	20		7		
Poa annua Bluegrass, annual	TS; TP	15-25; 10-30	10	28	Light, KNO_3 Light moisture	Prechill
Poa compressa Bluegrass, Canada	TS; TP	15-25; 10-30	10	28	Light, KNO_3 Light moisture	Prechill
Poa nemoralis Bluegrass, wood	TS; TP	15-25; 10-30	10	28	Light, KNO_3 Light moisture	Prechill
Poa palustris Bluegrass, fowl	TS; TP	15-25; 10-30	10	28	Light, KNO_3 Light moisture	Prechill

Kind of Seed	Substrata	Temperature (°C)	First Count (days)	Final Count (days)	Additional Directions	
					General Requirements	Fresh or Dormant Seeds
Poa pratensis Bluegrass, Kentucky	TS; TP	15-25; 10-30	10	28	Light, KNO_3 Light moisture	Prechill
Poa supina Bluegrass, supina	TP	15-25; 20-30	10	28	Light, KNO_3	Prechill
Poa trivialis Bluegrass, rough	TS; TP	15-25	10	21	Light, KNO_3 Light moisture	Prechill
Psathyrostachys juncea Wild-rye, Russian	TP	15-25	7	14	Light	Prechill
Pseudoroegneria spicata Wheatgrass, beardless	TP	15-25	7	14		Prechill; KNO_3
Alternate method for *Pseudoroegneria spicata*	TP	10-30	7	14	Light	Prechill; KNO_3
Puccinellia distans Alkaligrass, weeping	TP	15-25	10	28	Light, KNO_3 Light moisture	Prechill
Raphanus sativus Radish, garden	BP; RT	20	4	7		
Raphanus sativus var. *oleiformis* Radish oilseed or forage type	BP; RT	20	4	7		
Rheum x hybridum Rhubarb	TS; TP	20-30	7	28	Light	
Rumex acetosa Sorrel	TP; TS	20-30	3	14	Light	
Alternate method for *Rumex acetosa* Sorrel	TP; TS	15	7	14	Light	
Salvia officinalis Sage	BP; S; RT	20-30	7	14		
Satureja hortensis Savory	BP; TP; RT	20-30	5	21		
Satureja montana Savory	BP; TP; RT	20-30	5	21		
Secale cereale Rye	S; BP; RT	20		7		Prechill
Securigera varia Vetch, crown	BP; RT	20	7	14		
Setaria italica subsp. *italica* Millet, foxtail	BP; S; RT	20-30; 25	4	7		Sharp alternations of temperature
Setaria italica subsp. *italica* Millet, Italian	BP; S; RT	20-30; 25	4	7		Sharp alternations of temperature
Sinapis alba Mustard, white	TP; BP; RT	15-25; 25	4	10		Prechill; Light
Solanum melongena Eggplant	RP; TP BP; RT	20-30	7	14	Light moisture	Light, KNO_3
Sorghum bicolor Sorghum	S; BP; RT	20-30; 20-35	4	10		Prechill
Sorghum x drummondii Sorghum Sudan grass hybrids	S; BP; RT	20-30	4	10		Prechill
Sorghum x drummondii Sudangrass	S; BP; RT	20-30	4	10		Prechill
Spinacia oleracea Spinach	BP; RT	15; 10	7	21	Light moisture	Prechill

Kind of Seed	Substrata	Temperature (°C)	First Count (days)	Final Count (days)	Additional Directions	
					General Requirements	Fresh or Dormant Seeds
Taraxacum officinale Dandelion	TS; TP	20-30	7	21	Light See Section 4.14.3 Note 7	
Tetragonia tetragonoides Spinach, New Zealand	BP; RT	15	7	21	Soak fruits over-night (16 hours), air dry 7 hours; plant in very wet towels. Do not re-water unless later counts exhibit drying out.	On 21st day, scrape fruits and test for 7 additional days.
Thymus vulgaris Thyme	BP; TP; RT	15; 20; 20-30	7	21		
Tragopogon porrifolius Salsify	TS; BP; RT	15; 20	7	14	See Section 4.14.3 Note 7	Prechill
Trifolium aureum Clover, hop or yellow	BP; RT	20; 18	4	10		
Trifolium campestre Clover, large hop	BP; RT	20; 18	4	10		
Trifolium dubium Clover, small (suckling) hop	BP; RT	20; 18	4	10		
Alternate method for above *Trifolium* spp.	BP; RT	15	7	14		
Trifolium fragiferum Clover, strawberry	BP; RT	20; 18		5		Prechill
Trifolium hybridum Clover, alsike	BP; RT	20; 18		5		Prechill
Trifolium incarnatum Clover, crimson	BP; RT	20; 18		5		Prechill
Trifolium pratense Clover, red	BP; RT	20; 18		5		Prechill
Trifolium repens Clover, white (incl. Ladino)	BP; RT	20; 18		5		Prechill
Trifolium resupinatum Clover, Persian	BP; RT	20		7		Prechill
Alternate method for above *Trifolium* spp.	BP; RT	15		10		
Trifolium subterraneum Clover, subterranean	BP; RT	20; 18	4	10		
Alternate method for above *Trifolium* spp.	BP; RT	15	7	14		
x Triticosecale spp. Triticale	S; BP; RT	20		7		Prechill; Preheat
Triticum aestivum Wheat, common	S; BP; RT	20; 15		7		Prechill; Preheat
Triticum aestivum subsp. *spelta* Spelt	S; BP; RT	20; 15	7	12		Prechill; Preheat
Triticum turgidum subsp. *dicoccum* Emmer	S; BP; RT	20; 15	10	14		Prechill; Preheat
Triticum turgidum subsp. *durum* Wheat, durum	S; BP; RT	20; 15		7		Prechill; Preheat

Kind of Seed	Substrata	Temperature (°C)	First Count (days)	Final Count (days)	Additional Directions	
					General Requirements	Fresh or Dormant Seeds
Valerianella locusta Cornsalad	BP; RT	15	7	28		
Vicia faba Bean, horse	S; RT	20	10	14	Light; See Section 4.7.4	
Vicia faba Bean, broad	S	20	10	14	Light; See Section 4.7.4	Prechill
Vicia faba Bean, faba	S; RT	20	6	14	Light; See Section 4.7.4	
Vicia faba Bean, tick	S; RT	20	6	14	Light; See Section 4.7.4	
Vicia pannonica Vetch, Hungarian	S; BP; RT	20	5	10		
Vicia sativa subsp. *sativa* Vetch, common	S; BP; RT	20	5	10		
Vicia villosa subsp. v*illosa* Vetch, hairy	S; BP; RT	20	5	10		
Vigna radiata var. radiata Bean, mung	S; RT	25		8		
Vigna unguiculata subsp. *unguiculata* Cowpea	S; RT	20-30	5	8		
Zea mays subsp. *mays* Corn (open-pollinated, synthetic)	S; RT	25		7		
Zea mays subsp. *mays* Corn, hybrid	S; RT	25		7		
Zea mays subsp. *mays* Corn, pop or sweet	S; RT	25		7		

*A light intensity of 1000 lux or more may be required to induce complete germination.

4.7 SPECIAL GERMINATION PROCEDURES

4.7.1 *Pascopyrum smithii,* Western wheatgrass

a. Conduct the germination test by planting the Western wheatgrass on top of blotters moistened with 0.2% KNO_3. Germinate in the dark and do not prechill.

b. Western wheatgrass exhibits strong dormancy for some time after harvest. If it appears there may be fresh or dormant seeds remaining at the end of the germination test one of the following procedures should be used to assess the level of dormancy:

(i). Conduct a tetrazolium test (TZ) on remaining seeds and report percentage viable seeds as dormant.

(ii). Conduct a tetrazolium test on 200 seeds and estimate the percentage dormancy as follows:

The total viable seeds as determined by the TZ test - the total germinated seeds (normal + abnormal) = Estimated number of dormant seeds.

$$\frac{\text{estimated number of dormant seeds}}{\text{200 (based on the total seeds tested for TZ)}} \times 100 = \text{Estimated percent dormant seed}$$

Because some dormant seeds may die during the germination test, the tetrazolium test on 200 seeds is considered the more accurate estimate of dormancy.

c. Reporting procedure: The percent germination section of the report of analysis must show a dash (-). In "Remarks" the following statement should be made:

"Due to inherent dormancy in this species, the total of germination and dormant seed is to be used for grading purposes. Level of dormancy was determined by the tetrazolium test.

Germination	____ %
Dormant seed	____ %
Total germ + dormant	____ %

4.7.2 *Beta* spp

Wash for at least 4 hours in running water at a temperature of 20-25°C. If a beet seed washer is not used, the seeds may be soaked for the same period in still water, using at least 250 mL water for each 100 seeds, which must be changed as follows: every 15 minutes for the first hour, then every 30 minutes for the remaining three hours. After soaking is completed, remove the seeds from the water and drain for at least 60 minutes on a dry absorbent surface at a maximum temperature of 25°C. Plant on a substrate which has been thoroughly drained toremove all excess water (e.g. stand blotters on edge for at least 1/2 hour after soaking). For multigerm seed, frequent counts must be made (e.g. at 3, 5, 7 and 10 days) in order to keep track of the seedlings and avoid miscounts. See Section 4.10.6 a.

4.7.3 Phaseolus vulgaris, garden bean

If hypocotyl collar rot is observed on seedlings, the sample involved may be retested using a 0.3 to 0.6 percent calcium nitrate solution to presoak the substratum. The solution is prepared by dissolving 3 grams (for a 0.3% solution) to 6 grams (for a 0.6% solution) of $Ca(NO_3)_2$ in 1 litre of water.

4.7.4 *Vicia faba*, Broad Bean

The use of a 0.1% calcium nitrate solution in place of water to moisten the substratum prevents the blackening of seedlings. The solution is prepared by dissolving 1 gram of $Ca(NO_3)_2$ in 1 litre of water. The seeds should be well spaced (e.g. 10 seeds in sand in a 12 X 12 X 4 cm container). The seed must be well covered, with as much sand below as above the seed.

4.7.5 Coated Seeds (includes pelleted and encrusted seed

Germination tests on coated seed units and on de-coated seed must be conducted in accordance with methods in Table 5 (see definition of coated seed in section 3.10.1).

Coated seeds must be placed on the substratum in the condition in which they are received without rinsing, soaking, or any other pretreatment which will affect the coating material (except for mixtures, as noted below). Kinds for which soaking or washing is specified in Section 4.6.2 Table 5 (e.g. *Beta* spp.) must not be soaked or washed in the case of coated seed.

Coated seed units in mixtures which are colour coded or can otherwise be separated by kinds must be germinated as separate kinds without removing the coating material. Coated seed units in mixtures which cannot be separated by kind without removing the coating material must have the coating removed in a manner that will not affect the germination capacity of the seeds. The de-coated seeds must be planted as separate kinds on the same day the coating material is removed.

The moisture level of the substratum is important and may depend on the water-absorbing capacity of the coating material. A retest may be necessary before satisfactory germination of the sample is achieved. Symptoms of phytotoxicity may be evident when germinating coated seeds in paper substrata. In such cases a comparative test or retest in sand or soil-less organic growing media may be necessary.

4.7.6 Tetrazolium Testing

Methods for conducting tetrazolium tests on agricultural seeds are given in the current *Handbook on Tetrazolium Testing*, published by the International Seed Testing Association and/or the *AOSA Tetrazolium Testing Handbook* published by the Association of Official Seed Analysts. Tetrazolium test results may be used as the basis for grading of fall planted cereals, but must be confirmed by a standard germination test. For Western wheatgrass, a tetrazolium test is to be conducted to estimate the percentage dormant seeds (see Section. 4.7.1). For other crop kinds, tetrazolium test results are to be used for information only, not as the basis for grading. Results of tetrazolium tests are to be reported under "Remarks", or in a section designated for reporting of tetrazolium test results on the report of analysis.

4.7.7 Use of Fungicides

For grading purposes, seed for germination tests must not be treated with fungicide in the laboratory. However, when it is considered necessary to retest because of seed-borne disease present in the sample, or if the results are going to be used for information and not for grading, a suitable fungicide may be applied. The results of the untreated tests are to be reported, with the results of the treated tests being reported in the 'remarks' section of the report of analysis.

4.8 TREATMENTS FOR PROMOTING GERMINATION OF DORMANT SEEDS

Except in the case of certain species of the Fabaceae (see Section 4.10.7) and Western wheatgrass (see Section 4.7.1), dormant or hard seeds are not included when seed is graded in accordance with the germination standards of the Seeds Regulations. Therefore, in order to estimate the germination potential of a sample when dormant seeds are present, appropriate dormancy breaking methods must be used.

When dormancy is suspected in a sample, or when hard or dormant seeds remain at the end of the test period, the test or retest must include one of the treatments indicated in the "Fresh or Dormant seeds" column of Section 4.6.2 Table 5 and described in this section. **When a treatment is used as listed in the "Fresh or Dormant Seeds" column of Table 5 or an alternate method that is not listed for the kind under test in Table 5, its use must be indicated on the report of analysis**.

4.8.1 Prechilling

Prechilling is the exposure of imbibed seeds to low temperatures before being given the prescribed germination temperature. During the prechilling period, the seeds are held in an imbibed condition, in (or on) the regular germination substratum, at a constant temperature of not less than 5°C or more than 10°C and this temperature must be maintained within ± 2° C. In Section 4.6.2 Table 5 prechilling is indicated for those kinds of seeds for which experience has shown it to be effective in overcoming dormancy; prechilling may be tried, however, for other kinds of seeds in which dormancy is evident. Rechilling or midchilling may be used if many dormant seeds remain at the first or later counts.

The prechilling period is usually 3 to 5 days, but may be more or less if deemed appropriate. Rechilling is usually 2 days. The prechilling and rechilling periods are not included in the germination period specified in Table 5.

4.8.2 Potassium Nitrate

Instead of water, a 0.2% solution of potassium nitrate, prepared by dissolving 2g KNO_3 in one litre of water, is used to saturate the germination substratum at the beginning of the test. Water is used for subsequent moistening. If short roots occur (for example, as may occur in the *Poa* spp.) the strength of the solution may be reduced to 0.1%.

4.8.3 Predrying

Predrying of seeds having relatively high moisture content is often effective in hastening after-ripening, especially in freshly-harvested cereals. This procedure is particularly useful in years having adverse conditions during the maturing and harvesting season. The period of predrying is not included in the germination period specified in Section 4.6.2 Table 5.

a. Room temperature predrying. For species where dormancy is naturally of short duration, it is often sufficient to store the sample in a dry place for a short period. Spread the seed in shallow layers in open containers at normal room conditions for a period of up to 7 days.

b. Preheating. The replicates for germination are heated at a temperature not exceeding 35°C with free air circulation for a period of up to seven days before they are placed under the prescribed germination conditions. In some cases it may be necessary to extend the preheating period.

4.8.4 Gibberellic Acid

When the conventional dormancy breaking techniques are not completely effective, gibberellic acid may be used.

The germination substratum is moistened with a 500 ppm (0.05%) solution of GA_3, prepared by dissolving 0.5g GA_3 in one litre of water. When dormancy is weaker 200 ppm may be enough; when it is stronger up to 1000 ppm may be used. When a concentration higher than 800 ppm is required, the GA_3 must be dissolved in a phosphate buffer solution. The buffer solution is prepared by dissolving 1.7799 g of dibasic dihydrate sodium phosphate ($Na_2HPO_4.2H_2O$) and 1.3799 g of monobasic monohydrate sodium phosphate ($NaH_2PO_4.H_2O$) in one litre of distilled water.

4.8.5 Light

For dormant seeds, the light intensity should be approximately 750-1250 lux from cool white fluorescent lamps. The tests must be illuminated during at least eight hours in every 24 hour cycle and during the high temperature period when the seeds are germinated at alternating temperatures. Seeds for which light is prescribed should be germinated on top of the substratum.

4.9 COUNTS AND DURATION OF TEST

4.9.1 Counts

Seedlings may be counted when they have reached a stage of growth at which all essential structures can be evaluated.

a. **First counts.** The approximate number of days from planting to the first count is given in Section 4.6.2 Table 5. This is a guideline and deviations are permissible, depending on the development of the seedlings, and whether or not any pretreatments were given.

b. **Intermediate counts.** These may be made at the discretion of the analyst after the seedlings have reached a sufficient stage of growth for all essential structures to be evaluated. Intermediate counts should be conducted if continued growth of seedlings would hamper evaluation at the final count.

c. **Final counts.** The number of days to the final count is given in the "final count" column of Table 5. For permissible deviations, see Section 4.9.2 and 4.9.3.

d. For tests in sand or soil-less organic growing media lasting no more than 14 days, the first count may be omitted.

4.9.2 Early termination of tests

A test may be terminated only when germination is completed and only clearly abnormal seedlings and clearly dead and decaying seeds remain.

4.9.3 Extension of tests

A test may be extended under the following circumstances:

a. If the test has been prechilled or rechilled, the test may be extended by an equivalent number of days see Section 4.8.1.

b. . If a test is planted on a day of the week which results in the prescribed final count falling on a weekend or public holiday, the test may be extended to the first working day following.

c. If, at the prescribed final count,

(i). the number of seeds found to have germinated equals or exceeds the number found on the previous count, indicating a late wave of germination, and ungerminated seeds remain, or

(ii). there remains a number of fresh seeds or small seedlings which are difficult to evaluate (the test may be extended no more than one-half the number of days prescribed for final count to a maximum of five days).

(iii). the test has been extended this must be reported on the report of analysis

4.10 EVALUATION OF TESTS

4.10.1 Seedling Evaluation

The seedling descriptions of Section 4.14 must be followed to determine the classification of seedlings as normal or abnormal.

Seedlings which have reached a stage when all essential structures can be accurately assessed, and are normal, must be removed from the test at the first and any subsequent intermediate counts. Badly

decayed seedlings should be removed in order to reduce the risk of secondary infection, but abnormal seedlings with other defects must be left on the substratum until the final count.

4.10.2 Chemically Injured Seedlings

Seeds that have been over-treated with or accidentally exposed to certain pesticides may produce stunted seedlings having short, thickened roots, hypocotyls, epicotyls or coleoptiles. Such seedlings must be classified as "abnormal". If the number of seedlings classified as abnormal because of chemical damage could affect the grade of the sample, then a retest in soil-less organic growing media must be conducted.

4.10.3 Frozen and Immature Cereals

When a sample is characterized by seedlings on the borderline of abnormality as a result of frost damage, or by spindly seedlings from immature seeds, or by these two conditions together, a statement to this effect should be made on the report of analysis.

4.10.4 Seedlings Infected With Fungi or Bacteria

Seedlings which are affected by fungi or bacteria to the extent that essential structures are impaired, should be regarded as normal only when it is clearly evident that the parent seed is not the source of infection and it can be determined that all the essential structures are present. Samples difficult to assess due to the presence of fungi or bacteria should be retested, possibly with increased spacing or in sand or soil-less organic growing media.

Seeds which are obviously dead and mouldy and which may be a source of contamination of healthy seeds must be removed at each count and the number of such dead seeds should be recorded on the worksheet.

The presence of disease should be noted on the report of analysis, but identification of the disease must only be made by someone with appropriate training.

4.10.5 Borderline Seedlings

These are seedlings which are on the borderline between normal and abnormal. If there is only one such seedling, count it as normal. If there is an even number, count half of them as normal. If there is an odd number, count 2 out of 3, 3 out of 5, etc., as normal.

4.10.6 Two or More Seedlings from One Seed Unit

a. Multiple-seeded units: New Zealand spinach, *Beta* spp., schizocarps of the Apiaceae, multiple seeds of burnet, and seed units of grasses (*Dactylis glomerata, Poa* spp. oats, etc.) consisting of multiple florets, must be regarded as having germinated if they produce one or more normal seedlings. Only one seedling per multiple unit is to be counted.

b. Multiple embryos: The development of two or more embryos in one seed, a phenomenon known as polyembryony, occurs occasionally in many kinds of crop seeds and more frequently in a few (e.g. in Kentucky bluegrass). When such seeds produce at least one normal seedling, they are considered to have germinated and are included in the percentage of germination.

4.10.7 Hard Seeds

Hard seededness can be removed by mechanical scarification. Mechanical scarification is when the seed is cut, pierced, filed or sandpapered to improve permeability to moisture and gasses. Care must be taken to scarify the seed coat at a suitable place in order to avoid damaging the embryo and the resulting seedling. The best places are either immediately above the tips of the cotyledons or to the sides of the cotyledons.

The percentage of hard seeds is to be recorded and added to the percent germination for all legumes listed in Grade Tables VIII, IX and X and for sainfoin, hairy vetch and common vetch of Grade Table II.1. If fresh seeds of these kinds, or seeds which have just started to germinate are present at the end of the prescribed germination period, remove all hard seeds (record their number) and continue the test no more than one-half the number of days prescribed for final count to a maximum five days. The additional normal seedlings must be included in the percentage of germination.

4.11 CALCULATION AND REPORTING OF GERMINATION RESULTS

4.11.1 Calculation of results

The results of the tests are recorded on the worksheet as a number of normal, abnormal seedlings and un-germinated seeds for each replicate (see Section 4.3.4 and 4.10.7). If a result for any of the applicable categories is found to be nil, a “0” must be recorded.

The sum of the calculation for percentage by number of normal seedlings, abnormal seedlings and un-germinated seed must be 100. The percentages must be rounded to the nearest whole number (see Section 4.11.4 Rounding).

When a germination test was not undertaken, or a germination test component was not looked for, enter “-“ in the appropriate space on the Report of Analysis.

4.11.2 Single tests

When a single test is conducted and retesting is not required, the average of the replicates must be reported as percent germination or, for kinds listed in Section 4.10.7, percent germination plus hard seeds.

4.11.3 More than one test

When one or more retests are conducted for one of the reasons described in Section 4.12, the result to be reported must be in accordance with the directions of Section 4.12. When one or more retests or concurrent tests are made by either the same method, or an alternate method listed in Section 4.6.2 Table 5, for a reason other than described in Section 4.12, the results of all tests within one tolerance range, as given in Table 7, must be averaged and reported as the percentage of germination or germination plus hard seeds (see Section 4.10.7). When the results of such tests are not within tolerance of each other, a third test must be conducted and the average of compatible results must be reported. If the third test falls between the first two and is compatible with both, the average of all three must be reported.

4.11.4 Rounding

a. **The Result of a Germination Test** is calculated as the average of four 50 seed replicates, two or four 100 seed replicates (sub-replicates of 25 seeds are combined into 50 seed replicates). The percentage is calculated to the nearest whole number (0.5 is taken to the higher figure) except for values between 99.5% and 99.9% which must be dropped to 99%. The sum of the normal and abnormal seedlings and un-germinated seeds must be 100 percent. The percentage of abnormal seedlings, dead seeds, and hard seeds (see Section 4.10.7) is calculated in the same way.

(i). The percentage of normal seedlings is rounded to the nearest whole number, xx.5 is taken to the higher number (e.g. xx.0 to xx.49 is rounded down to xx; xx.5 and greater is rounded up to xx+1). The value for normal seedlings is not adjusted after this step.

(ii). Calculate the integer part of the remaining percentages, sum the values obtained. If the sum is 100, the rounding procedure ends, if not continue with the following steps.

(iii). For the percentage of abnormal seedlings, hard seeds (see Section 4.10.7) and dead seeds:

- Find the value with the greatest decimal part among the remaining percentages and round this percentage to the upper whole number, keep this value as a final result, calculate the integer part of the remaining percentages.
- Sum the values obtained.
- If the sum is 100, the procedure ends, if not continue with another step (i to iii).

In case of equal decimal parts, the priority order is abnormal seedlings, hard seeds (see 4.10.7) and dead seeds.

b. Pure Living Seed. Pure Living Seed is the percent pure seed multiplied by percent germination (i.e. PLS = %Pure seed X % Germination /100). For the grasses of Grade Tables XI and XII, pure living seed percentages must be rounded in the manner described in Section 4.11.4.a. for germination percentages. When calculating pure living seed, the rounded germination number and the percentage pure seed are multiplied to determine the percentage pure living seed, as in the following example: pure seed 95.8%, germination 86% (86.25 rounded), pure living seed 82% (82.38 rounded).

4.11.5 Reporting of results

All test results to be used for the grading of seed lots must be reported on a report of analysis as follows:

a. Section 1.0 Report of Analysis.

b. Additional reporting requirements, as applicable:

(i). When a modified method is used, the method must be clearly indicated. (See Section 4.2.2.e)

(ii). Mixtures of cereal seeds, Grade Table III, forage seeds, Grade Table XIII, lawn and turf, Grade Table XIV or ground cover, Grade Table XV for components to be named on the label report the percentage germination of each kind separately. (See Section 4.4.2.b and c)

(iii). When testing a kind of seed for which a method is not given in Section 4.6.2 Table 5 the method used must be clearly reported. (See Section 4.6.1)

(iv). *Pascopyrum smithii,* Western wheatgrass: the percent germination section of the report of analysis is to show a dash (-). In "Remarks" the following statement must be made (See Section 4.7.1.c):

"Due to inherent dormancy in this species, the total of germination and dormant seed is to be used for grading purposes. Level of dormancy was determined by the tetrazolium test.

Germination ____ %
Dormant seed ____ %
Total germ + dormant ____ % “

(v). Tetrazolium test: results are to be reported under "Remarks", or in a section designated for reporting of tetrazolium test results. (See 4.7.6)

(vi). When Fungicides are used, the results of the untreated tests are to be reported, with the results of the treated tests being reported in the 'remarks' section. (See Section 4.7.7)

(vii). When a treatment for promoting germination of dormant seeds is used as listed in the "Fresh or Dormant Seeds" column of Table 5 or an alternate method that is not listed for the kind under test in Table 5, its use must be indicated. (See Section 4.8)

(viii). When a test has been extended after the prescribed final count, the extension period must be reported. (See Section 4.9.3.c)

(ix). Frozen and Immature Cereals, when a sample is characterized by seedlings on the borderline of abnormality as a result of frost damage, or by spindly seedlings from immature

seeds, or by these two conditions together, a statement to this effect should be made. (See Section 4.10.3)

(x). Seedlings Infected With Fungi or Bacteria the presence of disease should be noted but identification of the disease must only be made by someone with appropriate training. (See Section 4.10.4)

(xi). Hard Seeds the percentage of hard seeds is to be added to the percent germination for all legumes listed in Grade Tables VIII, IX and X and for sainfoin, hairy vetch and common vetch of Grade Table II.1. (See Section 4.10.7)

(xii). Pure Living Seed is to be reported as a percentage calculated to the nearest whole number. (See Section 4.11.4.b)

c. The germination result, as a percentage germination or for kinds listed in Section 4.10.7, percent germination plus hard seeds calculated to the nearest whole number (0.5 is taken to the higher figure) except for values between 99.5% and 99.9% which must be dropped to 99%. The sum of the percentages reported must be 100. (See Section 4.10.7, 4.11.1, 4.11.2, 4.11.3 and 4.11.4.a)

4.12 RETESTING

In the following cases the germination test must be repeated using an appropriate method. Refer to Section 4.13 for the tolerance tables and descriptions of how to use them.

a. Retest when dormancy is suspected using a dormancy breaking method (see Section 4.8 and 4.6.2 Table 5). Report the best result achieved. Where a dormancy breaking method was used to conduct the initial test and the same or another dormancy breaking method was used to conduct the retest; in-tolerance results must be averaged.

b. Retest when the results are not reliable because of phytotoxicity or spread of fungi or bacteria. Report the best result achieved.

c. Retest when there are a number of seedlings which are difficult to evaluate. Report the best result achieved.

d. Retest when there is evidence of errors in test conditions, seedling evaluation or counting. Report the result of the retest.

e. Where the results of a retest do not support the reason/assumption for the retest (e.g. dormancy, test conditions); in tolerance tests should be averaged.

f. When the range between the replicates in the first test exceeds the maximum tolerated range given in Section 4.13.2 Table 6 a retest must be conducted. If the second result is compatible with the first (e.g. the difference between the tests does not exceed the tolerance indicated in Section 4.13.4 Table 7) the average of the two tests must be reported. If the second test result is not compatible with the first, a third test must be conducted. The average of compatible results must be reported. If the third test falls between the first two and is compatible with both, the average of all three tests must be reported.

4.13 GERMINATION TOLERANCES

In germination testing, tolerance is defined as the amount by which a result (i.e. test or replicate result) may differ from another result or from a specification, without it being attributed to a difference in seed quality. The tolerances given here are based on variation resulting from random sampling. The tolerance tables are used in accordance with the principles outlined in Sections 4.11.3 and 4.12

For crop kinds for which hard seeds are included with percent germination (Section 4.10.7) tolerances are checked using the normal seedlings plus hard seeds percentage.

4.13.1 Use of Table 6: Tolerated differences between replicates

This table gives the maximum range in germination percentage tolerable between replicates, allowing for random sampling variation only at 0.05 probability. A retest must be conducted when the range between replicates exceeds the tolerated range for the average percent germination (see Section 4.12.f).

For tests not exceeding 200 seeds when less than 100 seeds are planted to a replicate (e.g. 10, 25 or 50-seed replicates), the 50-seed replicate column of Section 4.13.2 Table 6 must be used for deciding whether a retest is necessary. Replicates of less than 50 seeds which were closest together in the germinator must be combined to form 50-seed replicates. **NOTE:** To use the 50-seed replicate column, the results must be converted to percentages before determining the tolerated range.

For tests of 400 seeds, replicates of less than 100 seeds which were closest together in the germinator must be combined to form 100-seed replicates.

To find the maximum tolerated range calculate the average percentage of the replicates, to the nearest whole number. Locate the average in columns A or B of the table and read the maximum tolerated range opposite in the appropriate column for 200 or 400 seed tests.

Example 1.
A germination test using 4 x 50 seeds gave replicate counts of 35, 40, 38, and 32 normal seedlings. To determine whether the range between the lowest and the highest result is within the tolerated range for the average percent germination, each replicate result must be expressed as a percentage, i.e. 70, 80, 76 and 64%. The average germination is 73%. The 4 x 50 replicate column of Table 6 shows the maximum tolerated range at 73% to be 25. The actual range between the highest (80%) and lowest (64%) replicate is 16 which is within the tolerated range and no retest is therefore necessary.

Example 2.
A germination test of 2 x 100 seeds gave replicate results of 87 and 72 for an average of 80% (79.5% rounded). To find the tolerated range, enter Table 6 at 80%. The permitted difference in a 200-seed test is 13; the actual range is 15. These results are not within tolerance; therefore the sample must be retested. The retest gave a result of 85%, with all replicates within tolerance, for an average result of 83% (82.5% rounded). From Section 4.13.4 Table 7, the permitted difference between tests is 7; the actual range is 5, therefore the tests are compatible and the average of the two (83%) is reported.

Example 3.
A germination test using 2 x 50 seeds gave replicate counts of 35 and 45 normal seedlings. To determine whether the range between the lowest and the highest result is within the tolerated range for the average percent germination, each replicate result must be expressed as a percentage; i.e. 70% and 90%. The average germination is 80%. The 2 x 50 replicate column of Table 6 shows the maximum tolerated range at 80% to be 18. The actual range between the highest (90% and the lowest 70%) replicate is 20. The replicates are not within tolerance and therefore a retest is necessary. The retest gave a result of 85%, with all replicates within tolerance, for an average result of 83% (82.5% rounded). From Section 4.13.4 Table 7, the permitted difference between tests is 10; the actual range is 5, therefore the tests are compatible and the average of the two (83%) is reported.

4.13.2 Table 6. Maximum tolerated ranges in germination percentages of replicates or deciding when to retest.

Average Percent Germination		Number of 50 seed replicates		Number of 100 seed replicates	
		2	4	2	4
-A-	-B-	-C-	-D-	-E-	-F-
99	**2**	5	7	4	5
98	**3**	7	8	5	6
97	**4**	8	10	6	7
96	**5**	9	11	6	8
95	**6**	10	13	7	9
94	**7**	11	14	8	10
93	**8**	12	15	8	10
92	**9**	12	15	9	11
91	**10**	13	16	9	11
90	**11**	13	17	9	12
89	**12**	14	18	10	12
88	**13**	15	18	10	13
87	**14**	15	19	11	13
86	**15**	15	20	11	14
85	**16**	16	20	11	14
84	**17**	16	21	11	14
83	**18**	17	21	12	15
82	**19**	17	22	12	15
81	**20**	17	22	12	15
80	**21**	18	22	13	16
79	**22**	18	23	13	16
78	**23**	18	23	13	16
77	**24**	19	24	13	17
76	**25**	19	24	13	17
75	**26**	19	24	14	17
74	**27**	19	25	14	17
73	**28**	20	25	14	17
72	**29**	20	25	14	18
71	**30**	20	25	14	18
70	**31**	20	26	14	18
69	**32**	21	26	14	18
68	**33**	21	26	15	18
67	**34**	21	26	15	18
66	**35**	21	26	15	19
65	**36**	21	27	15	19
64	**37**	21	27	15	19
63	**38**	21	27	15	19
62	**39**	22	27	15	19
61	**40**	22	27	15	19
60	**41**	22	27	15	19
59	**42**	22	27	15	19
58	**43**	22	28	15	19
57	**44**	22	28	15	19
56	**45**	22	28	15	19
55	**46**	22	28	15	20
54	**47**	22	28	16	20
53	**48**	22	28	16	20
52	**49**	22	28	16	20
51	**50**	22	28	16	20

Tolerances are calculated from Table G1, *Handbook of Tolerances,* S.R. Miles, Proceedings of the International Seed Testing Association Vol. 28, No. 3, 1963. (Two-way test at 0.025 probability level).

4.13.3 Use of Table 7: Tolerated Differences Between Tests

In accordance with the principles outlined in Section 4.11.3, Section 4.13.4 Table 7 indicates the maximum range in germination percentages tolerable between different tests on the same submitted sample, in the same laboratory, allowing for random sampling variation only at 0.05 probabilities.

If the results of two tests are found to be compatible, the average of the tests is reported. To calculate the final reported result, the replicate results of all compatible tests are averaged. If the two tests are not compatible conduct a third test. Report the average of the replicates of the compatible tests (see Section 4.12.f).

Example 1
A germination test using 2 x 100 seeds gives a result of 75%. A retest on 2 x 100 seeds from the same sample gives a germination of 85%. The average of the two tests is 80%. Enter Section 4.13.4 Table 7 at 80% under column E (200-seed tests); the maximum tolerated range is 8. Since the difference between the two tests exceeds this range, a third test must be conducted. If the third test gave a result of 82% it would be compatible with both the first test (average 79%, range 7, tolerance 8) and the second test (average 84%, range 3, tolerance 7), so the average of all three (81%) would be reported. If the third test gave a result of 87%, it would not be compatible with the first test (average 81%, range 12, tolerance 7) but would be compatible with the second test (average 86%, range 2, tolerance 7) and so the average of the second and third tests (86%) would be reported (see Section 4.12.f).

Example 2
A germination test using 2 x 50 or 4 x 25 seeds gives a result of 70%. A retest on 2 x 50 or 4 x 25 seeds from the same sample gives a germination of 80%. The average of the two tests is 75%. Enter Section 4.13.4 Table 7 at 75% under column C (100-seed tests) the maximum tolerated range is 12. Since the difference between the two tests does not exceed this range the average of the tests are reported. To calculate the final reported result, the replicate results of all compatible tests are averaged (see Section 4.12.f).

4.13.4 Table 7.Maximum tolerated differences in germination percentages of tests for deciding which tests to average.

Average Percent Germination		Number of Tests							
		100 seed tests		200 seed tests			400 seed tests		
		2	3	2	3	4	2	3	4
A	B	C	D	E	F	G	H	I	J
99	2	3	4	2	3	3	1	2	2
98	3	4	5	3	3	4	2	2	3
97	4	5	6	3	4	4	2	3	3
96	5	5	7	3	4	5	3	3	3
95	6	6	7	4	5	6	3	3	4
94	7	7	8	4	5	6	3	4	4
93	8	7	8	5	6	6	3	4	4
92	9	7	9	5	6	7	4	4	5
91	10	8	9	5	7	7	4	5	5
90	11	8	10	6	7	8	4	5	5
89	12	9	10	6	7	8	4	5	5
88	13	9	11	6	7	8	4	5	6
87	14	9	11	6	8	8	4	5	6
86	15	9	11	7	8	9	5	6	6
85	16	10	12	7	8	9	5	6	6
84	17	10	12	7	8	9	5	6	6
83	18	10	12	7	9	9	5	6	7
82	19	10	13	7	9	10	5	6	7
81	20	11	13	7	9	10	5	6	7
80	21	11	13	8	9	10	5	6	7
79	22	11	13	8	9	10	5	6	7
78	23	11	14	8	9	10	5	7	7
77	24	11	14	8	10	11	6	7	7
76	25	12	14	8	10	11	6	7	8
75	26	12	14	8	10	11	6	7	8
74	27	12	14	8	10	11	6	7	8
73	28	12	14	8	10	11	6	7	8
72	29	12	15	9	10	11	6	7	8
71	30	12	15	9	10	11	6	7	8
70	31	12	15	9	10	11	6	7	8
69	32	13	15	9	11	12	6	7	8
68	33	13	15	9	11	12	6	7	8
67	34	13	15	9	11	12	6	8	8
66	35	13	15	9	11	12	6	8	8
65	36	13	16	9	11	12	6	8	8
64	37	13	16	9	11	12	6	8	8
63	38	13	16	9	11	12	6	8	8
62	39	13	16	9	11	12	6	8	9
61	40	13	16	9	11	12	6	8	9
60	41	13	16	9	11	12	6	8	9
59	42	13	16	9	11	12	7	8	9
58	43	13	16	9	11	12	7	8	9
57	44	13	16	9	11	12	7	8	9
56	45	13	16	9	11	12	7	8	9
55	46	13	16	9	11	12	7	8	9
54	47	14	16	9	11	12	7	8	9
53	48	14	16	9	11	13	7	8	9
52	49	14	16	9	11	13	7	8	9
51	50	14	16	9	11	13	7	8	9

Based on 5% probability levels calculated from Table G1, Handbook of Tolerances, S.R. Miles, Proc. Int. Seed Test. Assoc. Vol. 28, No.3, 1963.

4.14 SEEDLING DESCRIPTIONS

Seedlings, in general, have the following essential structures necessary for the continued development of the seedling (although some structures may not be visible in all species at the time of seedling evaluation).

- root system, consisting of primary and/or secondary, seminal or adventitious roots
- hypocotyl
- epicotyl
- cotyledon(s)
- terminal bud
- primary leaves

Seedlings with defects to these structures, as described in the abnormal seedling descriptions, are judged to be incapable of continued growth.

The detailed seedling descriptions assume that test conditions were adequate to allow proper assessment of the essential seedling structures. If it is suspected that the test conditions have contributed to seedling abnormalities or the spread of infection to the point where evaluation is difficult, the sample must be retested under more favourable conditions.

The "General Description" for each group of crop kinds describes a seedling without defects. While such a seedling is clearly normal, seedlings with some defects may also be classified as normal, provided the defects do not impair the functioning of the structure. The "Abnormal Seedling Description" is to be followed when judging the severity of defects. The seedling diagrams in this section are taken from the 2017 Edition of Association of Official Seed Analysts (AOSA) Rules for Testing Seeds, Volume 4. Seedling Evaluation.

4.14.1 Aizoaceae, Carpetweed Family

Tetragonia tetragonoides, New Zealand spinach

GENERAL DESCRIPTION

Seedling type: Epigeal dicot.

Food reserves: Leaflike cotyledons and perisperm.

Shoot system:
The hypocotyl elongates carrying the cotyledons above the media surface. The epicotyl usually does not show any development within the test period.

Root system: A primary root; secondary roots may develop within the test period.

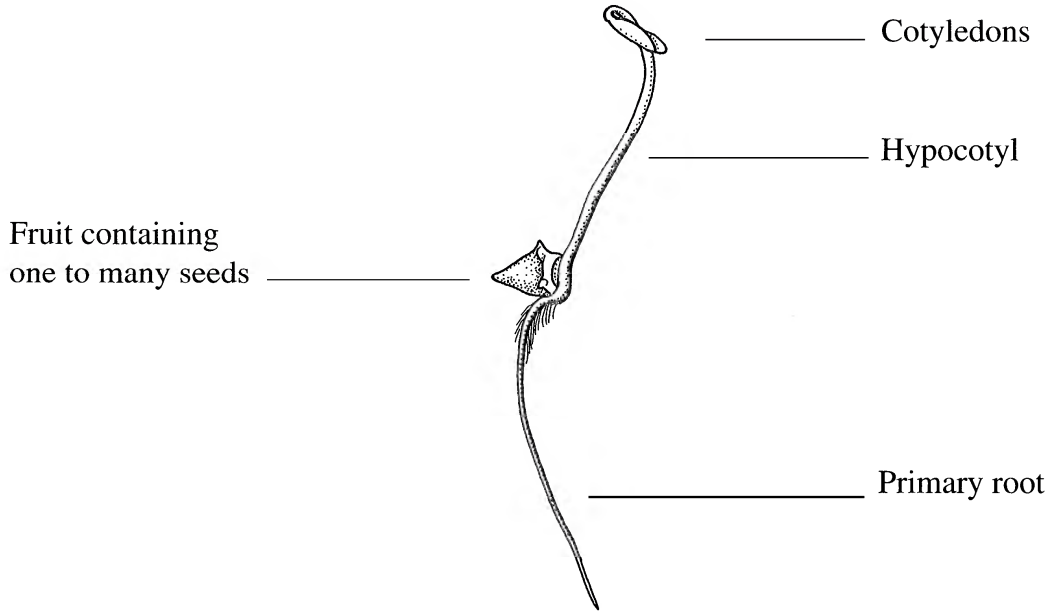


Fig.1 New Zealand Spinach

Abnormal Seedling Description

Cotyledons
- less than half of the original cotyledon tissue remaining attached.
- less than half of the original cotyledon tissue free of necrosis or decay.

Epicotyl:
- missing (may be assumed to be present if cotyledons are intact).

Hypocotyl:
- deep open cracks extending into the conducting tissue.
- malformed, such as markedly shortened, curled or thickened.
- watery.

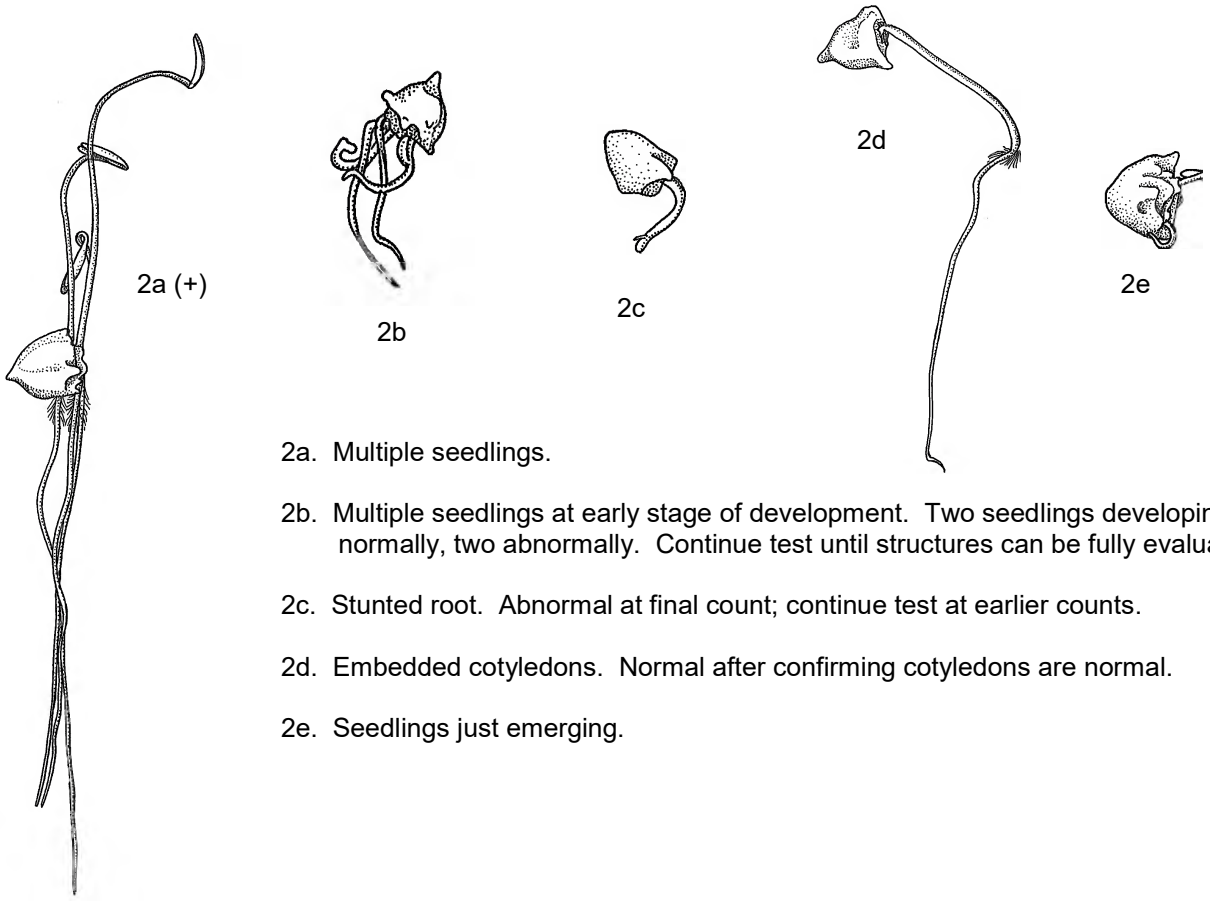
Root:
- none.
- weak, stubby or missing primary root with weak secondary or adventitious roots.

Seedling:

- one or more essential structures impaired as a result of decay from primary infection.
- albino.

Notes:

None



2a. Multiple seedlings.

2b. Multiple seedlings at early stage of development. Two seedlings developing normally, two abnormally. Continue test until structures can be fully evaluated.

2c. Stunted root. Abnormal at final count; continue test at earlier counts.

2d. Embedded cotyledons. Normal after confirming cotyledons are normal.

2e. Seedlings just emerging.

Fig. 2 New Zealand Spinach

4.14.2 Asteraceae, Sunflower Family I - Lettuce

Latuca sativa, lettuces, Celtus/Celtus

General Description

Seedling type: Epigeal dicot.

Food reserves: Cotyledons which expand and become thin, leaf-like and photosynthetic. Some varieties develop elongated petioles at the base of the cotyledons.

Shoot system: The hypocotyl elongates and carries the cotyledons above the media surface. The epicotyl usually does not show any development within the test period.

Root system: A long primary root.

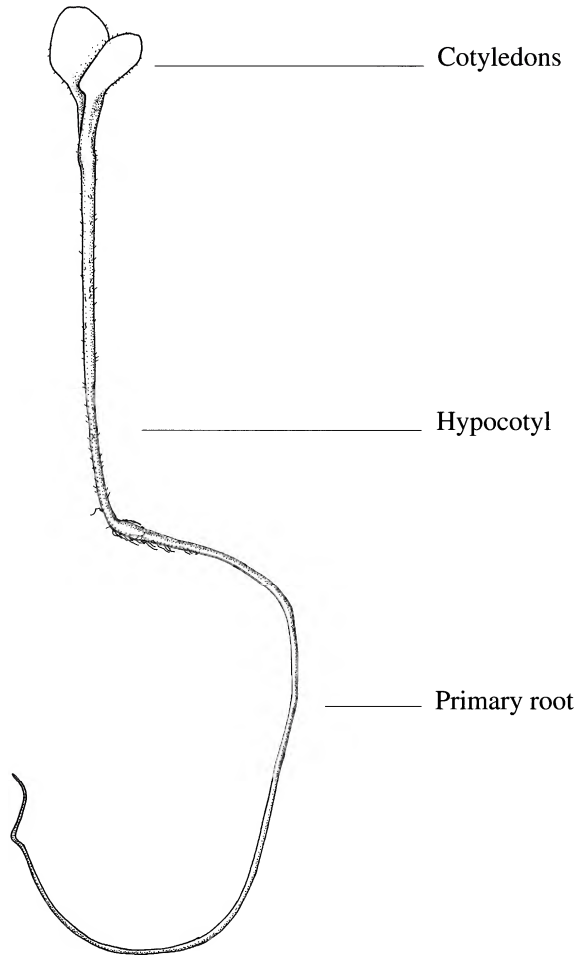


Fig 1. Lettuce

Abnormal Seedling Description

Cotyledons
- less than half of the original cotyledon tissue remaining attached.
- less than half of the original cotyledon tissue free of necrosis or decay (see notes 5 and 6).
- with any degree of physiological necrosis * (also see notes 5 and 6).

Epicotyl
- missing (may be assumed to be present if cotyledons are intact).
- any degree of necrosis or decay.

Hypocotyl
- deep open cracks extending into the conducting tissue.
- severely twisted or grainy.
- watery.

Root
- none.
- primary root tip blunt, swollen and discoloured.
- primary root with splits or lesions.

Seedling
- swollen cotyledons associated with extremely short or vestigial hypocotyl and root.
- one or more essential structures impaired as a result of decay from primary infection.
- albino.

Notes

***** The requirement to be free of any degree of physiological necrosis differs from the AOSA description, in which only seedlings with more than 50% of the cotyledons being necrotic are considered abnormal.

1. Toxic materials in the substrate will cause short, blunt roots.

2. Seedlings grown on top of white filter paper will be shorter than those on blue blotters.

3. Remove attached seed coats for seedling evaluation.

4. Seedlings with slight dormancy or light sensitivity may be slow to germinate.

5. Necrosis is manifested on lettuce cotyledons by softened grey, brown, black or reddish areas appearing adjacent to the midrib and lateral veins. This must not be confused with natural pigmentation of some varieties, or with insect damage.

   Physiological necrosis is associated with the connecting tissue and is indicated by discoloration from the main rib down to the terminal bud region at the junction of the cotyledons. It is often accompanied by shortened hypocotyls and roots, with the seed coat frequently remaining attached to the cotyledons.

   Seedlings showing any degree of physiological necrosis must be classified as abnormal. In "Remarks" indicate the percentage of necrotic seedlings (this percentage must include necrotic seedlings which are also otherwise abnormal).

6. Seedlings with extensive physiological necrosis on the cotyledons may be slower in growth than those without such affected areas. Hypocotyl and root length may be affected by other factors such as proximity to light, delayed germination or dormancy.

2a. Grainy hypocotyl.

2b. Shortened hypocotyl.

2c. No hypocotyl development, stubby root.

2d. Physiological necrosis.

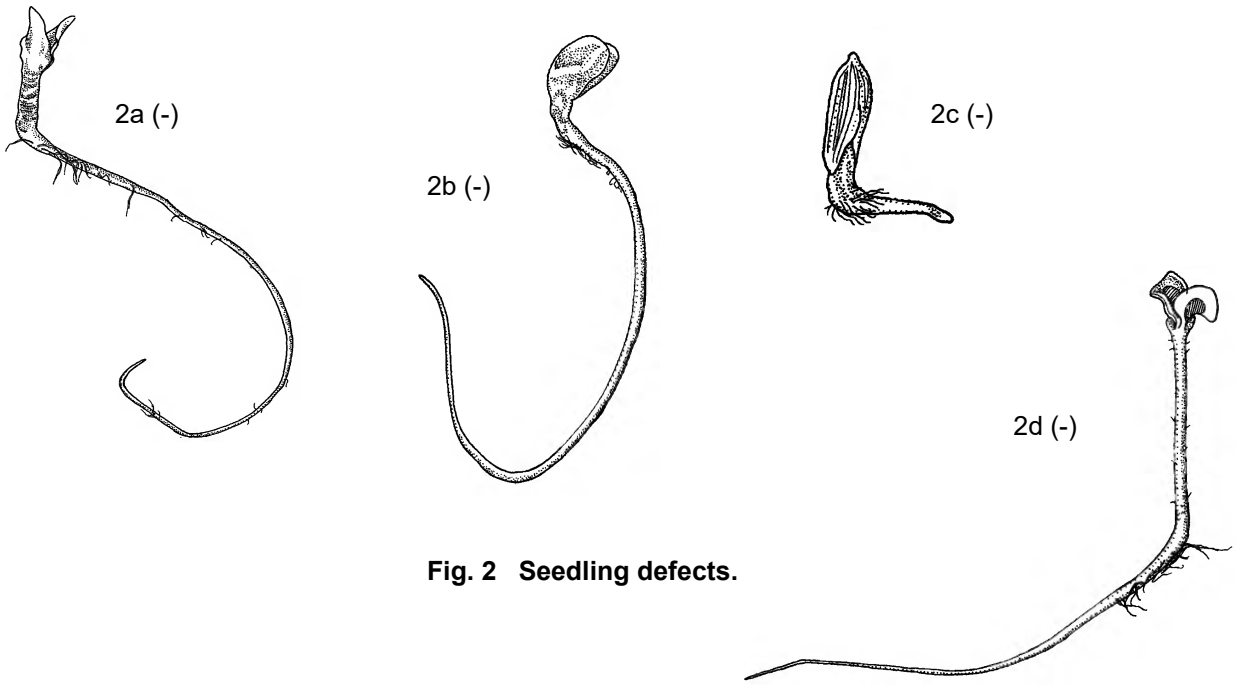


Fig. 2 Seedling defects.

4.14.3 Asteraceae, Sunflower Family II - Kinds Other Than Lettuce

Carthamus tinctorius, safflower
Cichorium endivia, endive
Cichorium intybus, chicory
Cynara cardunculus, artichoke, cardoon
Helianthus annuus, sunflower
Taraxacum officinale, dandelion
Tragopogon porrifolius, salsify

General Description

Seedling type: Epigeal dicot.

Food reserves: Cotyledons which expand and become thin, leaf-like and photosynthetic.

Shoot system: The hypocotyl elongates and carries the cotyledons above the media surface. The epicotyl usually does not show any development within the test period.

Root system: A long primary root with secondary roots usually developing within the test period.

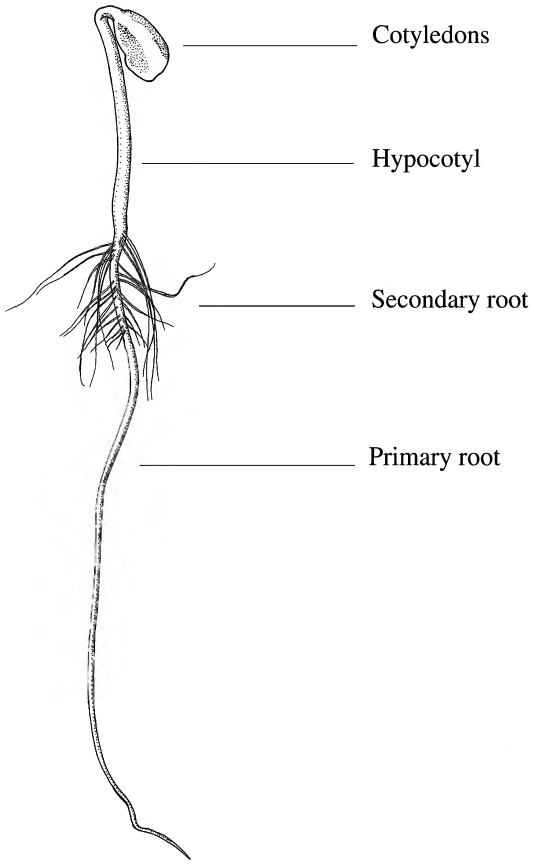


Fig. 1 Sunflower

Abnormal Seedling Description

Cotyledons
- less than half of the original cotyledon tissue remaining attached.
- less than half of the original cotyledon tissue free of necrosis or decay (see note 7).

Epicotyl
- missing (may be assumed to be present if cotyledons are intact).

Hypocotyl
- decayed at point of attachment.
- deep open cracks extending into the conducting tissue.
- malformed, such as markedly shortened, curled or thickened.
- watery.

Root
- none.
- weak, stubby or missing primary root with weak secondary or adventitious roots (see notes 1 and 5).

Seedling
- one or more essential structures impaired as a result of decay from primary infection.
- albino.

Notes:

1. Substrate with insufficient moisture may result in slow or abnormal development, bound roots, retarded secondary root development or unshed seed coats.

2. Some seed lots of sunflower will exhibit dormancy if the substrate is on the wet side.

3. Due to the thick, dry seed coat, imbibition may be slow and the subsequent germination erratic. Some seeds may just be starting at the end of the test period and it may be necessary to extend the test as allowed under Section 4.9.3.

4. All seeds in this group may exhibit some dormancy and a retest using appropriate dormancy breaking procedures may be necessary.

5. Frequently the root may become bound within the hard seed coat. If left in the test until the final count such seedlings may develop secondary roots sufficient to be considered normal. Bound roots are usually not a problem in soil-less organic growing media tests since the secondary root development is faster than in artificial media.

6. The hypocotyl may be slow to develop in seedlings with a damaged primary root.

7. Seedlings with unshed seed coats may have decayed cotyledons. The seed coat must be removed for evaluation.

8. For dormant samples of endive, add about 4 mm of water at the beginning of the test and remove excess water after 24 hours.

Fig. 2 Root defects.

2a. Normal seedling.

2b. Stubby primary root, sufficient secondary roots.

2c. Stubby primary root, insufficient secondary roots.

Fig. 3 Hypocotyl defects.

3a. Deep hypocotyl lesion

3b. Primary infection of hypocotyl.

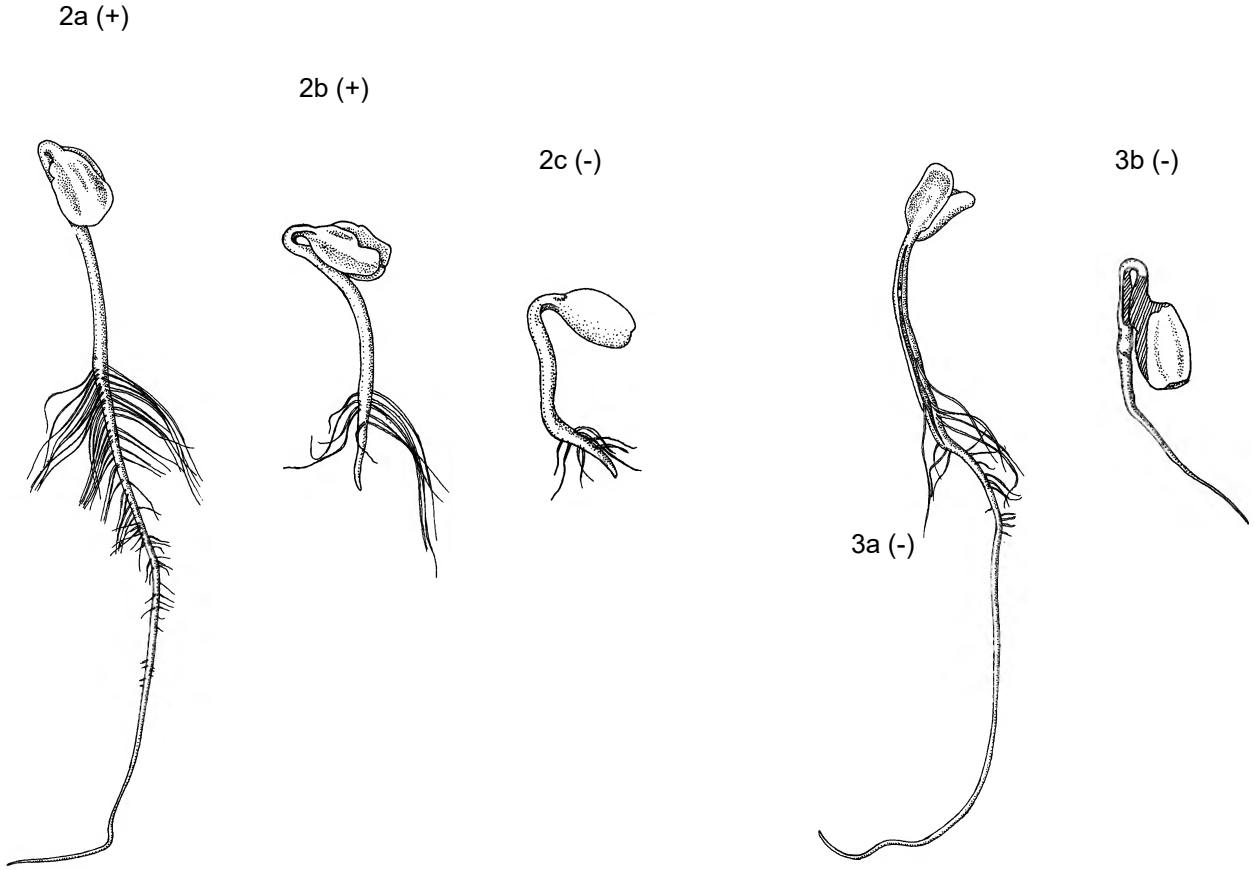
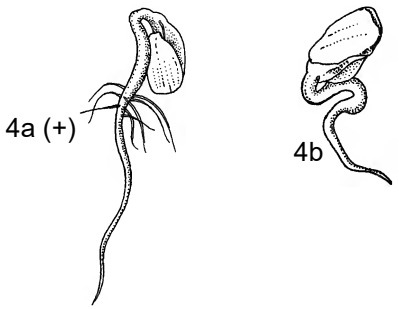


Fig. 4 Small seedlings.

4a. Late-germinating

4b. Seedling too small



4.14.4 Brassicaceae, Mustard Family

Brassica spp., mustards, etc.
Lepidium sativum, garden cress
Nasturtium officinale, watercress
Raphanus sativus, radish
Sinapis alba, white mustard

General Description

Seedling type: Epigeal dicot.

Food reserves: Cotyledons which expand and become thin, leaf-like and photosynthetic. In *Brassica*, *Sinapis* and *Raphanus*, the cotyledons are bi-lobed and folded, with the outer cotyledon being larger than the inner.

Shoot system: The hypocotyl elongates and carries the cotyledons above the media surface; the epicotyl usually does not show any development within the test period.

Root system: A long primary root.

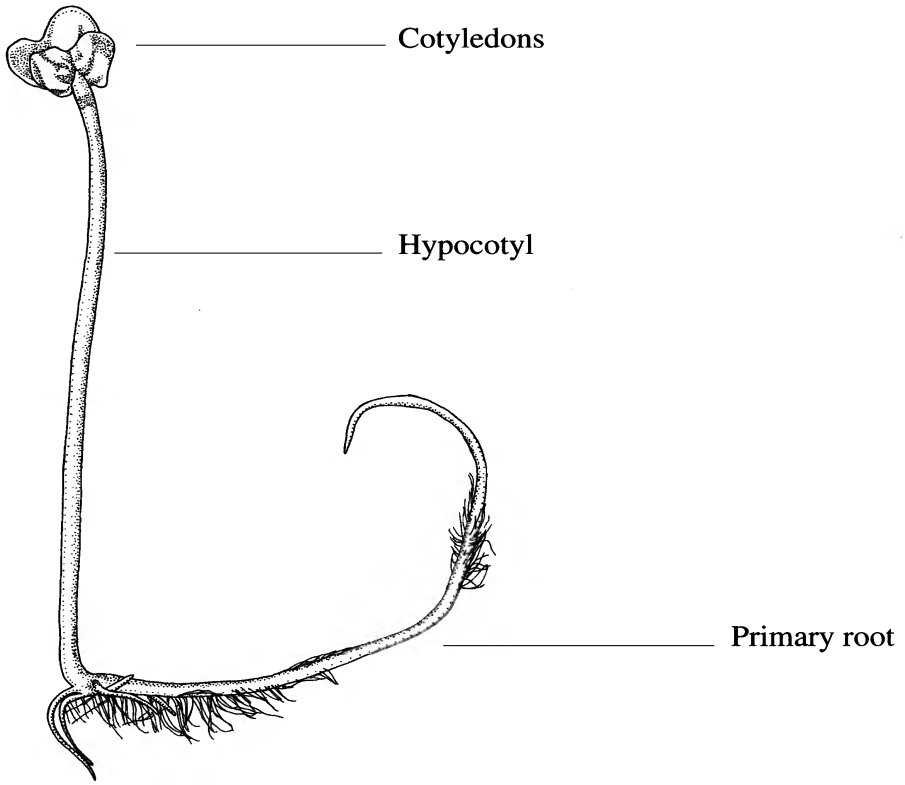


Fig. 1 Brassica

Abnormal Seedling Description

Cotyledons

- decayed at point of attachment.
- less than half of the original cotyledon tissue remaining attached.
- less than half of the original cotyledon tissue free of necrosis or decay.
- in seedlings of *Brassica*, one-half or more of the total cotyledon tissue yellow or white, with no green tint * (also see Note 1).

Epicotyl

- missing (may be assumed to be present if the cotyledons are intact).

Hypocotyl

- deep open cracks extending into the conducting tissue.
- malformed, such as markedly shortened, curled or thickened.
- watery.

Root

- weak, stubby or missing primary root (secondary roots will not compensate for a defective primary root).

Seedling

- one or more essential structures impaired as a result of decay from primary infection.
- albino.

* The evaluation of yellow cotyledons in *Brassica* differs from the AOSA description, which does not specifically describe this condition.

Notes:

1. Seedlings of *Brassica* with 50% or more of the total cotyledonary tissue entirely yellow, with no green tint, are to be classified as abnormal. This requires that the germination test be conducted with light to allow chlorophyll production in the cotyledons. Seedlings which have their seed coats attached at final count must not be considered to be abnormal due to yellow cotyledons. If it is apparent that the sample has a yellow cotyledon problem and there are a significant number of seedlings with their coats attached, then the test must be extended to allow shedding of the coats, or the sample must be retested using higher intensity light. In assessing seedlings with yellow cotyledons, analysts must make a rapid decision by scanning the replicate and removing obviously yellow seedlings, then continuing the evaluation without re-considering the first assessment of yellow. The assessment of yellow cotyledons may be aided by using a colour reference (e.g. the yellow cotyledons of a *Brassica* seedling which has not been exposed to light). If there is any hint of green in the yellow area, then the seedling must not be classified as abnormal due to the yellow cotyledon condition.

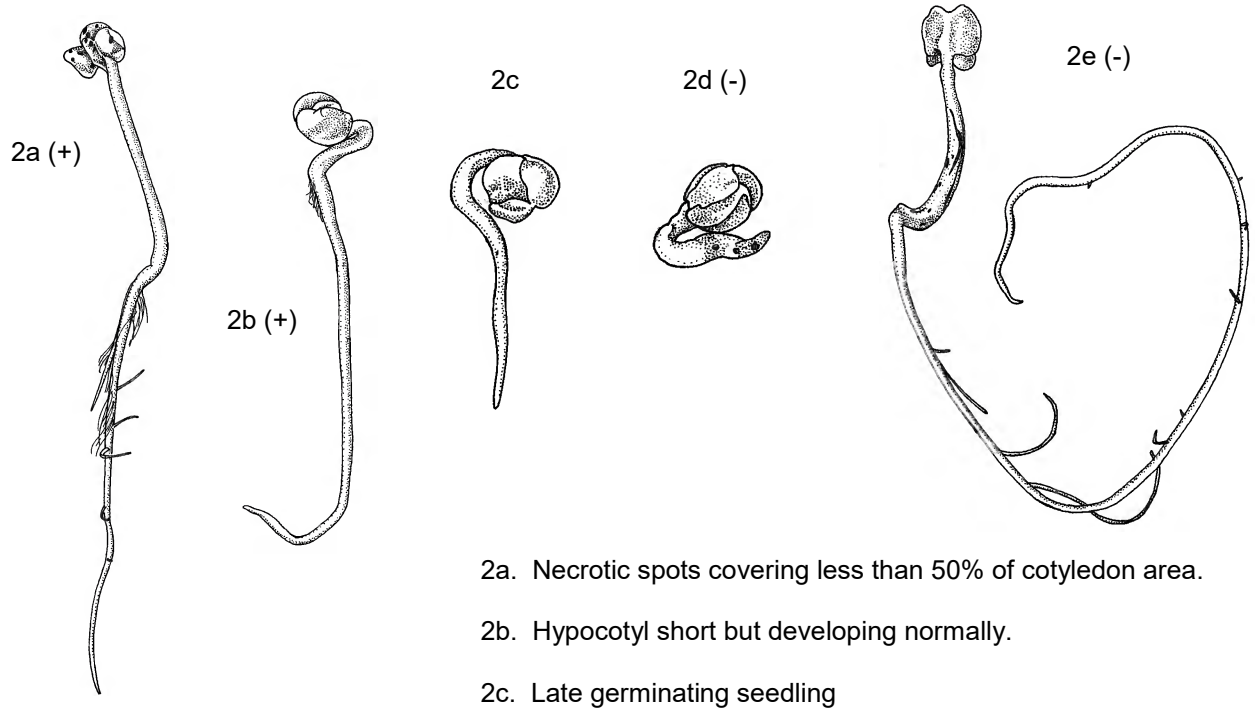


Fig 2. Brassica

2a. Necrotic spots covering less than 50% of cotyledon area.

2b. Hypocotyl short but developing normally.

2c. Late germinating seedling

2d. Stubby primary root, poor hypocotyl development

2e. Hypocotyl lesions.

4.14.5 Cannabaceae, Hemp Family

Cannabis sativa, hemp

General Description

Seedling type: Epigeal dicot.

Food reserves: Cotyledons which expand and become leaf-like and photosynthetic.

Shoot system: The hypocotyl elongates carrying the cotyledons above the media surface. The epicotyl usually does not show any development within the test period.

Root system: A primary root with root hairs; secondary roots may occasionally develop within the test period.

Abnormal Seedling Description

Cotyledons:
- less than half of the original cotyledon tissue remaining attached.
- less than half of the original cotyledon tissue free of necrosis or decay.

Epicotyl:
- missing (may be assumed to be present of the cotyledons are intact).

Hypocotyl:
- malformed, such as markedly shortened, curled or thickened.
- deep open cracks extending into the conducting tissue.
- watery.

Root:
- none.
- missing or stubby primary root with weak secondary or adventitious roots.

Notes:

None

4.14.6 Chenopodiaceae, Goosefoot Family

Beta vulgaris, beet, sugar beet, mangel, Swiss chard
Spinacea oleracea, spinach

General Description

Seedling type: Epigeal dicot.

Food reserves: Leaf-like cotyledons and perisperm.

Shoot system: The hypocotyl elongates carrying the cotyledons above the media surface. The epicotyl usually does not show any development within the test period.

Root system: A primary root; secondary roots may develop within the test period.

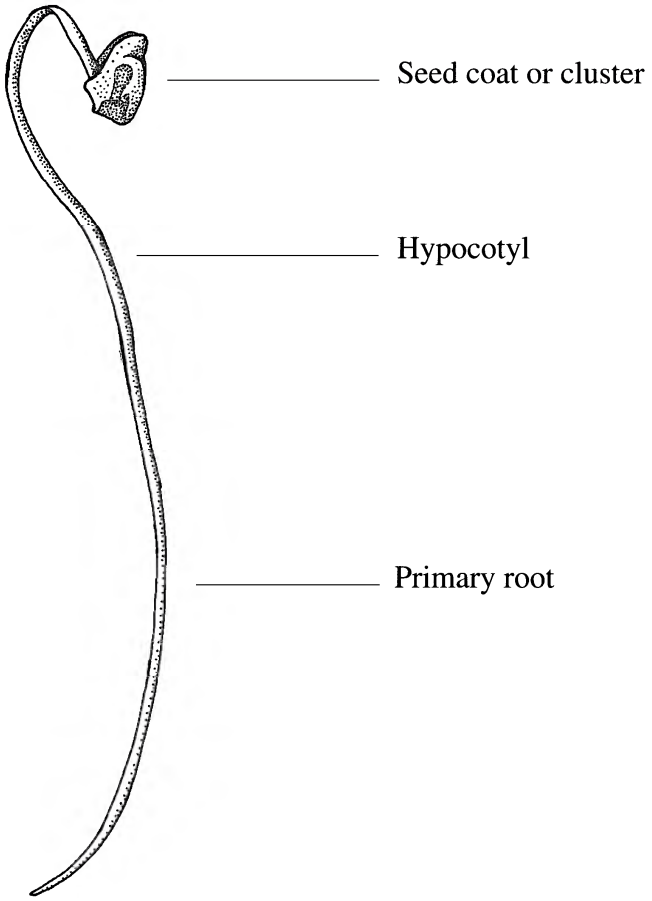


Fig. 1 Beet

Abnormal Seedling Description

Cotyledons:
- less than half of the original cotyledon tissue remaining attached.
- less than half of the original cotyledon tissue free of necrosis or decay.

Epicotyl:
- missing (may be assumed to be present if cotyledons are intact).

Hypocotyl:
- deep open cracks extending into the conducting tissue.

- malformed, such as markedly shortened, curled or thickened.
- watery.

Root:

- none.
- weak, stubby or missing primary root with weak secondary or adventitious roots.

Seedling:

- one or more essential structures impaired as a result of decay from primary infection (for discoloured seedlings of *Beta* spp., see note 2).
- albino.

Notes:

1. See Section 4.7.2 for directions to wash samples of *Beta* prior to planting. Chemical inhibitors in the cluster or seed coat may work together with excess water to rob the embryo of oxygen and thus prevent germination. It is important, therefore, to ensure the seeds or clusters are dried before planting.

2. Toxic substances from the clusters of *Beta* may cause discolouring of the hypocotyl and/or root. Seedlings which are slightly discoloured are to be classified as normal; however, if there is excessive discoloration, retest in soil-less organic growing media or by washing in running water for 3 hours.

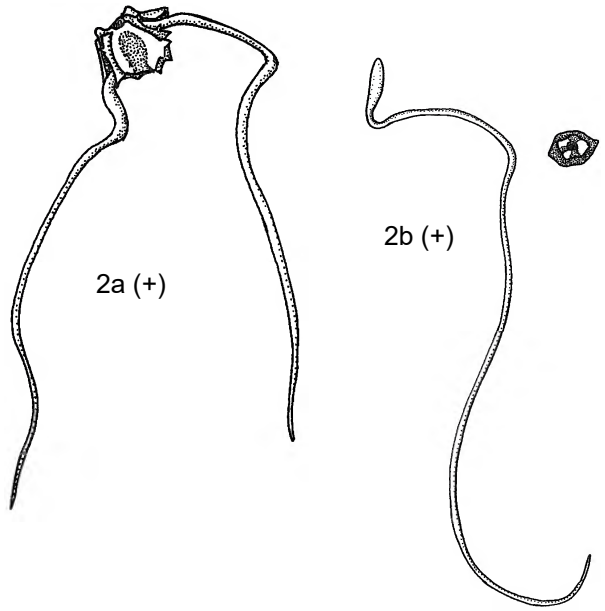
3. Frequent counts must be made on multigerm beet since the growing seedlings will separate from the cluster making it difficult to identify its source. Any cluster which produces at least one normal seedling is classified as normal; only one normal seedling per cluster is to be counted. (See Section 4.7.2 and 4.10.6)

Fig. 2

2a. Multiple seedlings.
2b. Seedling separated from cluster

Fig. 3 Small seedlings

3a. Late germinating seedling
3b. Stubby root
3c. No root development



3a (+) 3b (-) 3c (-)

4.14.7 Cucurbitaceae, Cucurbit Family

Citrullus lanatus var. *citroides*, citron
Citrullus lanatus var. *lanatus*, watermelon
Cucumis melo, melon or cantaloupe
Cucumis anguria, gherkin
Cucumis sativus, cucumber
Cucurbita spp., pumpkin and squash (winter and summer)

General Description

Seedling type: Epigeal dicot.

Food reserves: Cotyledons which are large and fleshy; they expand, become photosynthetic and are usually persistent beyond the seedling stage.

Shoot system: The hypocotyl elongates and the cotyledons are pulled free of the seed coat, which often adheres to a peg-like appendage at the base of the hypocotyl. The epicotyl usually does not show any development within the test period.

Root system: A long primary root with numerous secondary roots.

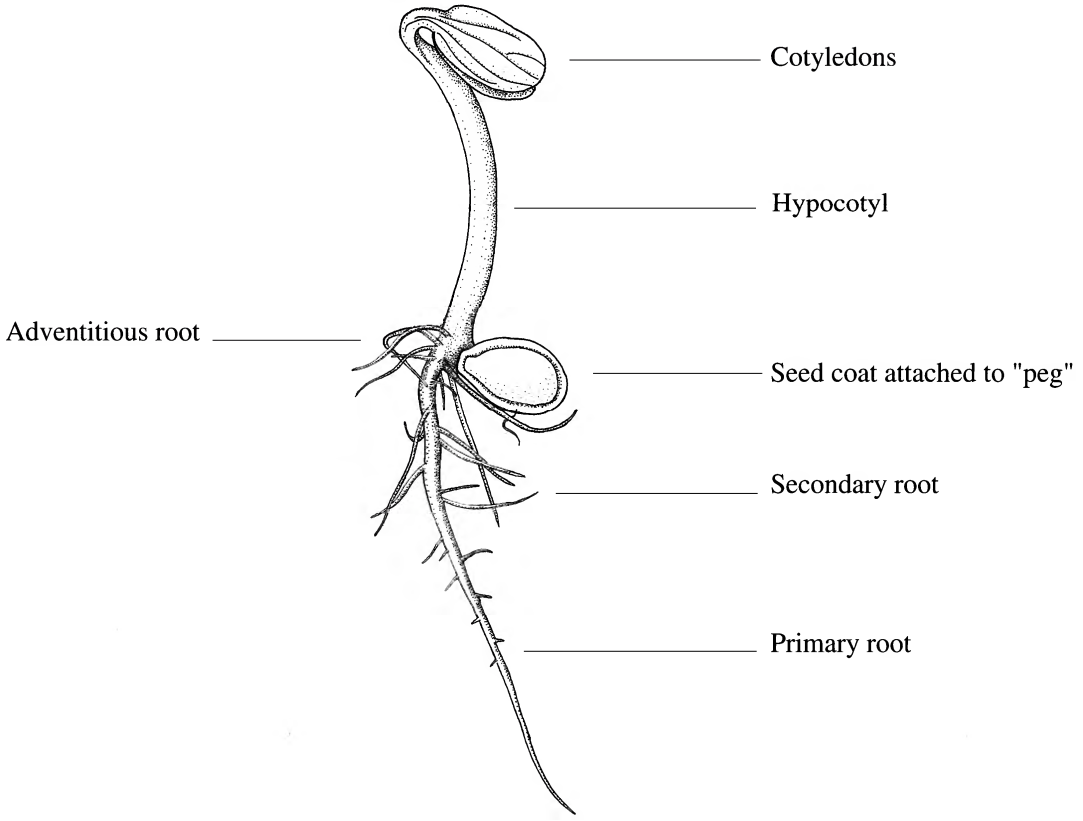


Fig. 1 Pumpkin

Abnormal Seedling Description

Cotyledons:
- less than half of the original cotyledon tissue remaining attached.
- less than half of the original cotyledon tissue free of necrosis or decay (see note 3).

Epicotyl:
- missing (can be assumed to be present if the cotyledons are intact).

Hypocotyl:

- deep open cracks extending into the conducting tissue.
- malformed, such as markedly shortened, curled or thickened (see note 2).

Note: The short, thickened area (or "peg") between the roots and the hypocotyl is a normal development.

Root:

- none.
- weak, stubby or missing primary root, with less than two strong secondary or adventitious roots.

Seedling:

- one or more essential structures impaired as a result of decay from primary infection.
- albino.

Notes:

1. In general, seedling development is best when substrata are kept on the dry side. Extra moisture may then need to be added at the time of the first count.

2. Samples must be retested in sand or soil-less organic growing media if there is evidence of chemical injury (characterized by badly thickened and shortened hypocotyls and roots). Seedlings showing chemical injury symptoms in the retest are to be classified as abnormal.

3. Seedlings with unshed seed coats may have decayed or damaged cotyledons. The seed coat must be removed for evaluation of the cotyledons.

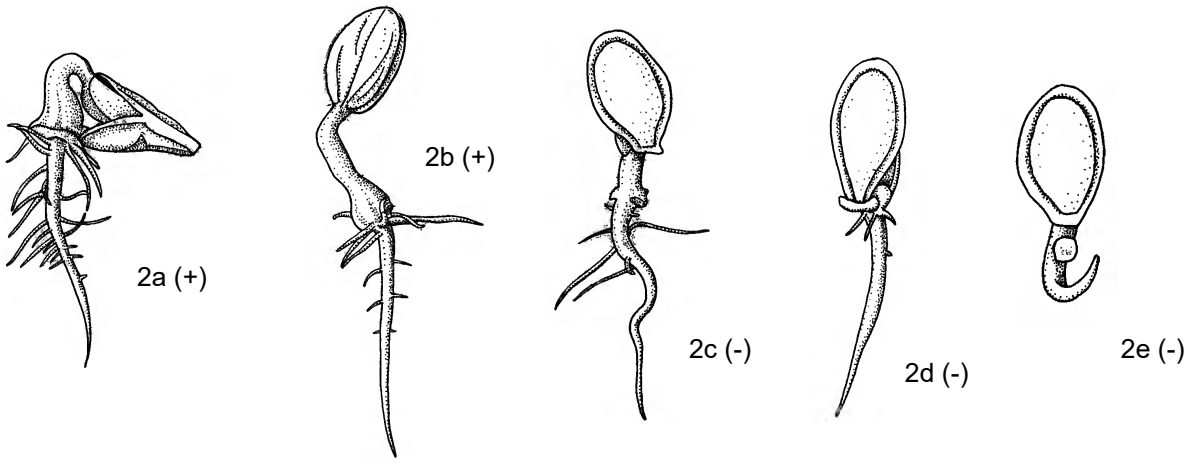


Fig. 2 Small seedlings.

2a. Late-germinating seedling

2b. Hypocotyl just long enough.

2c. Hypocotyl too short.

2d. No hypocotyl development.

2e. No hypocotyl, stubby root.

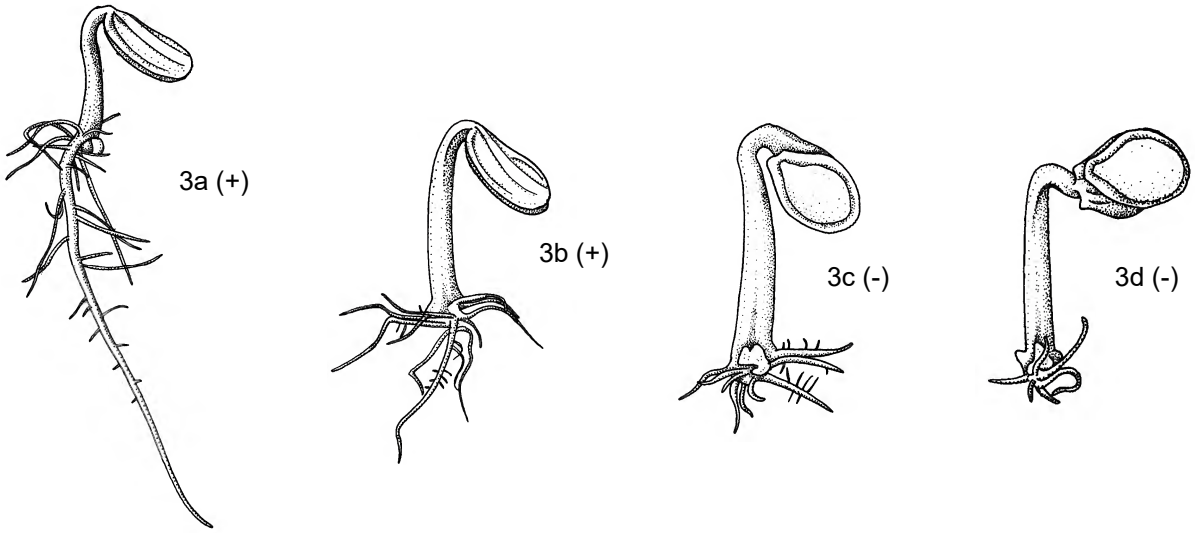


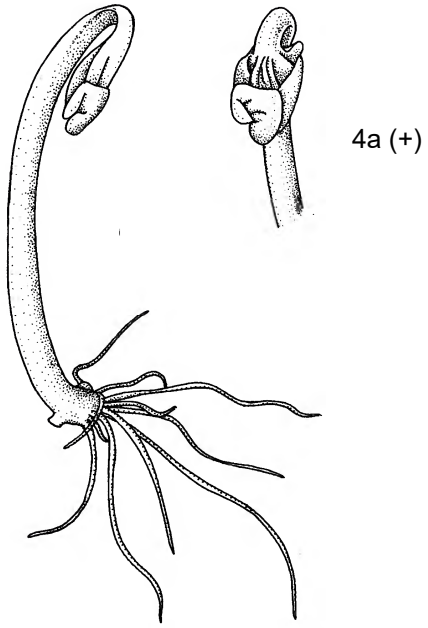
Fig. 3 Root defects.

3a. Normal development.

3b. Primary root missing or damaged, with sufficient secondary or adventitious roots.

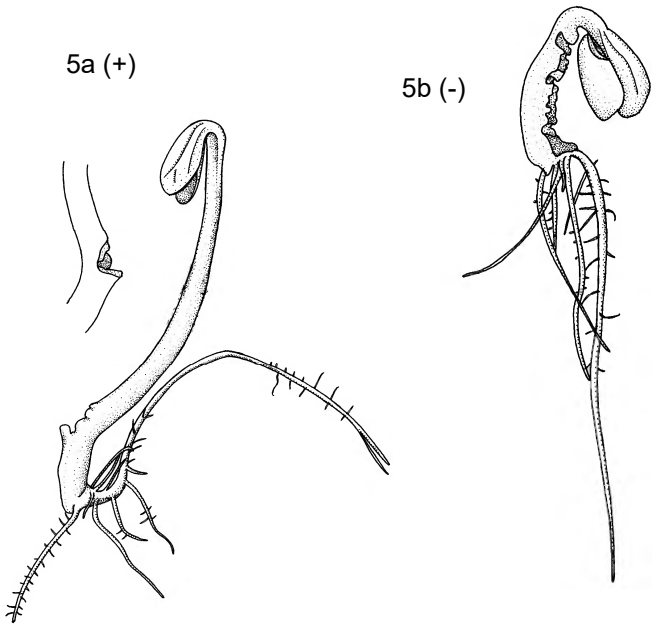
3c. Primary root missing or damaged, with insufficient secondary or adventitious roots.

3d. Insufficient roots.



4a. Cotyledons present but convoluted.

Fig. 4 Deformed cotyledons.



5a. Break caused by test conditions.
5b. Deep lesion.

Fig. 5 Hypocotyl lesions.

4.14.8 Fabaceae, Legume Family I - Large-Seeded Epigeal, Except Soybean and Lupine

Phaseolus vulgaris, garden bean and field bean
Phaseolus lunatus, Lima bean
Vigna radiata var. radiata, mung bean
Vigna unguiculata subsp. unguiculata, cowpea

NOTE: For purposes of these rules, a garden bean (*Phaseolus vulgaris*) variety is defined as one which is grown for its fleshy pod to be eaten. Other beans, including field beans, are defined as those grown for their seeds to be eaten. Beans which are grown for either pod or seed to be eaten are to be considered garden beans, and the requirements for cotyledons apply (see abnormal seedling description).

General Description

Seedling type: Epigeal dicot.

Food reserves: Cotyledons which are large and fleshy; some photosynthesis may occur, but this is a minor function. They shrivel and drop off when the food reserves are depleted.

Shoot system: The hypocotyl elongates and carries the cotyledons above the media surface. The epicotyl elongates causing the terminal bud to emerge from between the cotyledons; the primary leaves expand rapidly.

Root system: A long primary root with secondary roots.

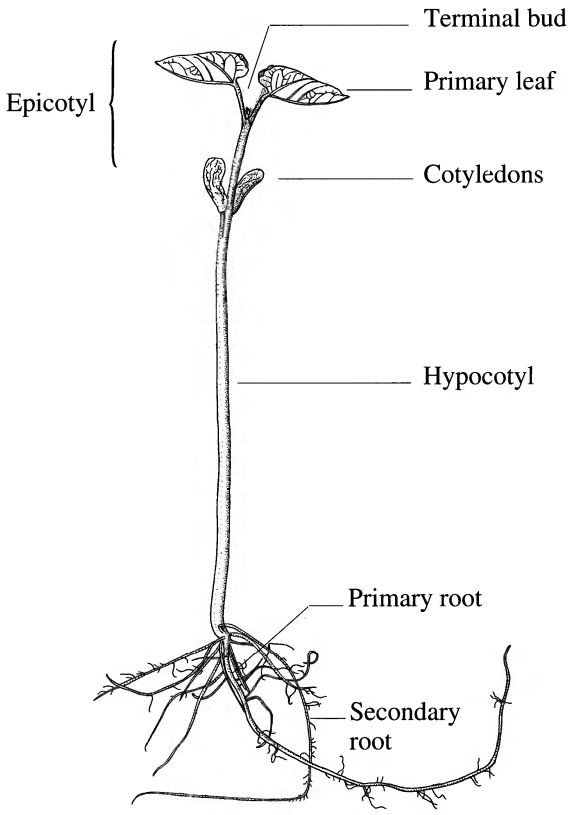


Fig. 1 Bean.

Abnormal Seedling Description

Cotyledons:

Garden bean (*Phaseolus vulgaris*, in part):

- less than half of the original cotyledon tissue remaining attached.
- less than half of the original cotyledon tissue free of necrosis or decay.

All others:

- cotyledons are not assessed. EXCEPTION: If both cotyledons are missing and the seedling is generally weak, then the seedling is considered abnormal.

Epicotyl:

- missing.
- deep, open cracks.
- malformed, such as markedly curled or thickened.
- less than one primary leaf.
- primary leaves too small in proportion to the rest of the seedling, usually associated with visible defects of, or damage to, the main stem of the epicotyl.
- terminal bud missing or damaged (see note 6).

Hypocotyl:

- deep open cracks extending into the conducting tissue (see notes 1 and 4).
- malformed, such as markedly shortened, curled or thickened.
- (see also note 3.)

Root:

- none.
- weak, stubby or missing primary root with weak secondary or adventitious roots (see note 5).

Seedling:

- one or more essential structures impaired as the result of decay from primary infection (but see note 6).
- albino.

Notes:

1. Towels rolled too tightly may cause constriction of growing seedlings, resulting in malformation. Tight rolls, often in combination with a mid-test watering, may cause hypocotyl cracking or splitting.

2. Seeds of beans must be well spread out on or in the substrate. In general, the larger the seed, the more space it needs. Tightly concentrated seeds may compete to their detriment for water and for space to expand.

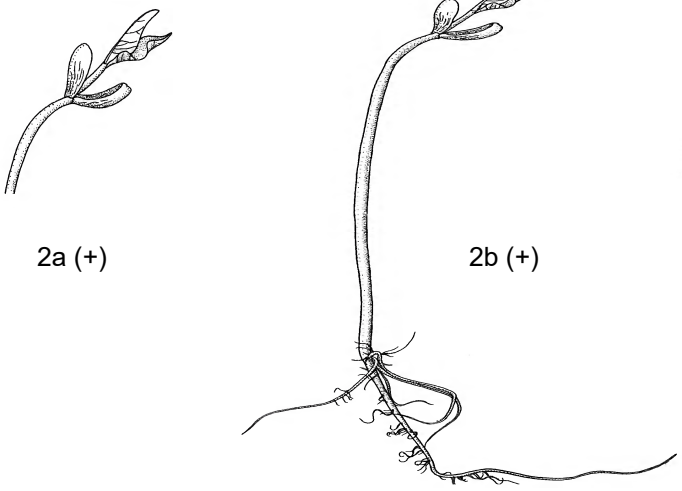
3. Hypocotyl collar rot is a breakdown in hypocotyl tissue characterized by "water-soaking" and collapse of the hypocotyl below the cotyledonary node. The lesion area later becomes discoloured, shrivelled and necrotic. The condition is recognized as a laboratory phenomenon caused by insufficient calcium available to the seedling. If hypocotyl collar rot is observed on seedlings of garden beans, the sample involved must be retested using a 0.3 to 0.6 percent calcium nitrate solution to presoak the substratum (see Section 4.7.3).

4. A healed break in the hypocotyl, sometimes referred to as a "knee", is to be considered an allowable defect.

5. A seedling with the root bound within a tough seed coat is to be considered normal.

6. If a few seedlings with total or partial decay to the epicotyl are found, they may be classified as normal, provided the hypocotyl and root are normal. The epicotyl on such seedlings usually does not decay when grown in a fairly dry environment and is exposed to light. Retests, preferably in soil-less organic growing media or sand, will aid in interpretation of such seedlings.

7. Large-seeded legumes are especially susceptible to threshing or combine damage. Seed which has been mechanically damaged may produce seedlings with damaged primary roots, hypocotyls or epicotyls, or broken or detached cotyledons. Bruised areas are usually necrotic or decayed. Damage at the point of attachment of the cotyledons may be difficult to evaluate if seedlings are removed too early in the test period.



2a (+) 2b (+) 2c (-) 2d (-)

2a. Leaves large enough.

2b. Leaves large enough.

2c. Leaves borderline size, with damage to the epicotyl.

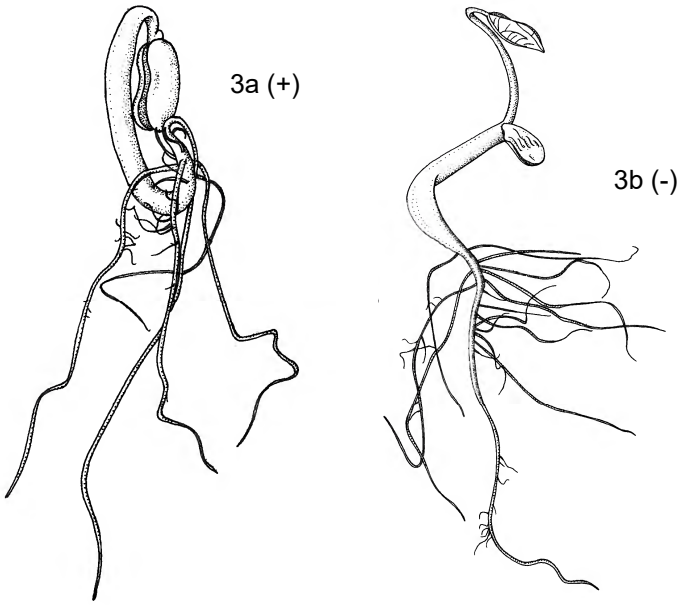
2d. Leaves too small.

Fig. 2 Leaf size.

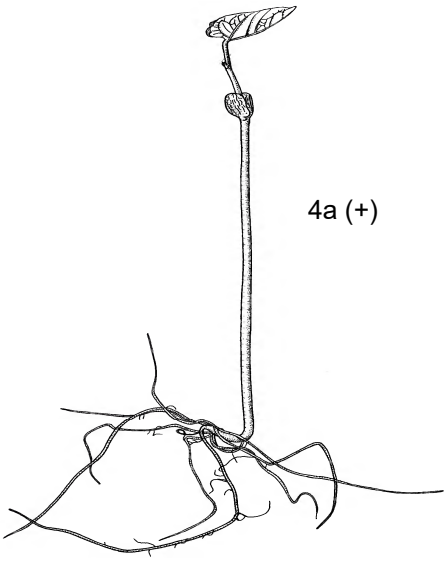
Fig. 3 Thickened hypocotyl.

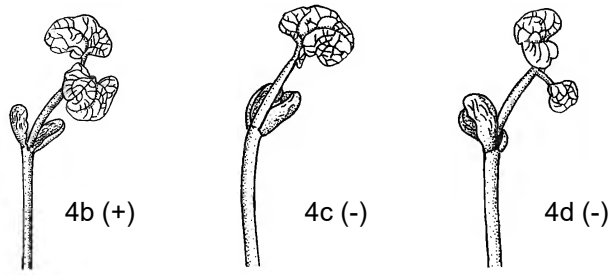
3a. Hypocotyl thickened due to towel test.

3b. Hypocotyl thickened and short relative to epicotyl.



3a (+) 3b (-)





4a. One leaf missing, remaining leaf and terminal bud undamaged.

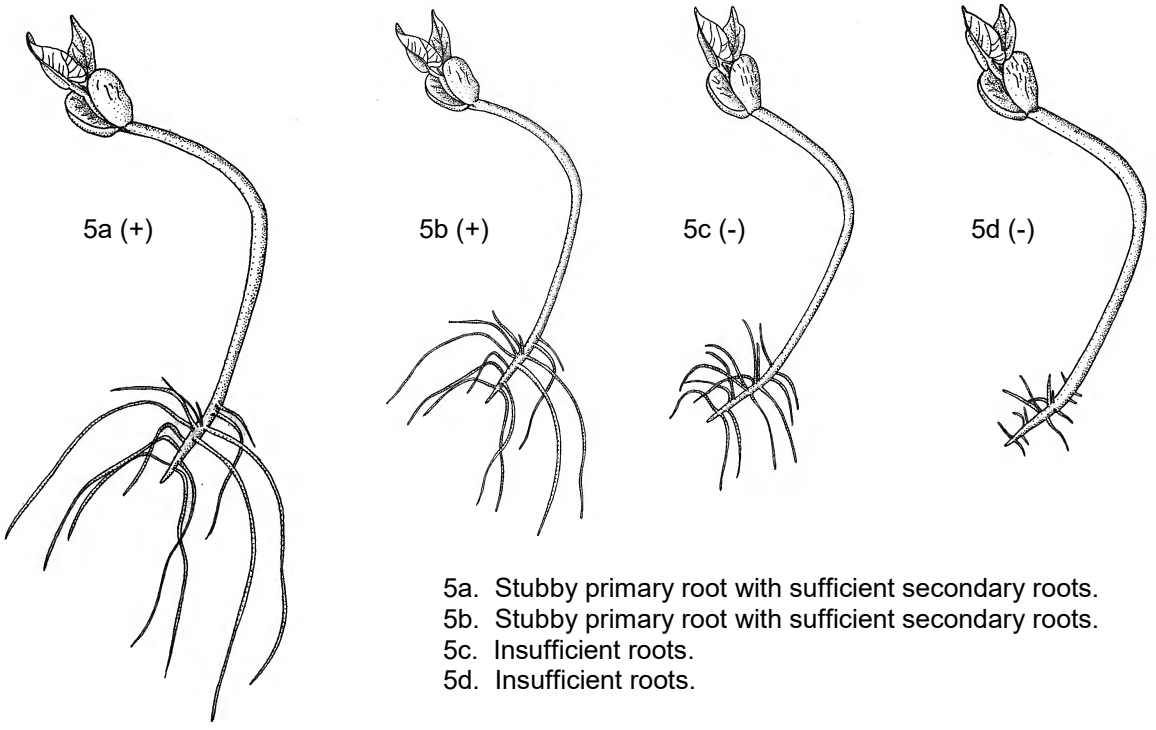
4b. Two leaves damaged but proportional in size to the rest of the seedling.

4c. One leaf missing, the remaining leaf damaged.

4d. Two leaves damaged and too small in proportion to the rest of the seedling.

Fig. 4 Damaged primary leaves.

Fig. 5 Root defects.



5a. Stubby primary root with sufficient secondary roots.
5b. Stubby primary root with sufficient secondary roots.
5c. Insufficient roots.
5d. Insufficient roots.

Fig. 6 Cotyledons, bean other than garden bean.

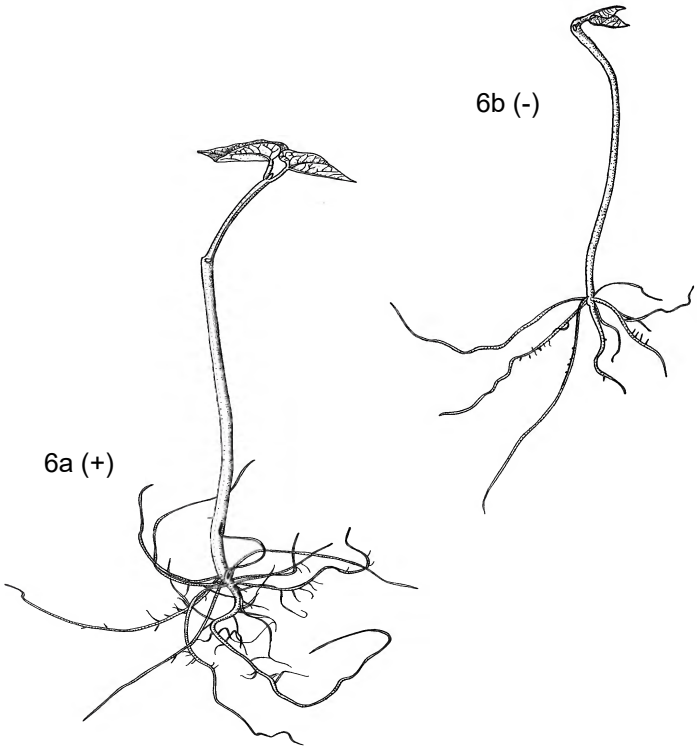
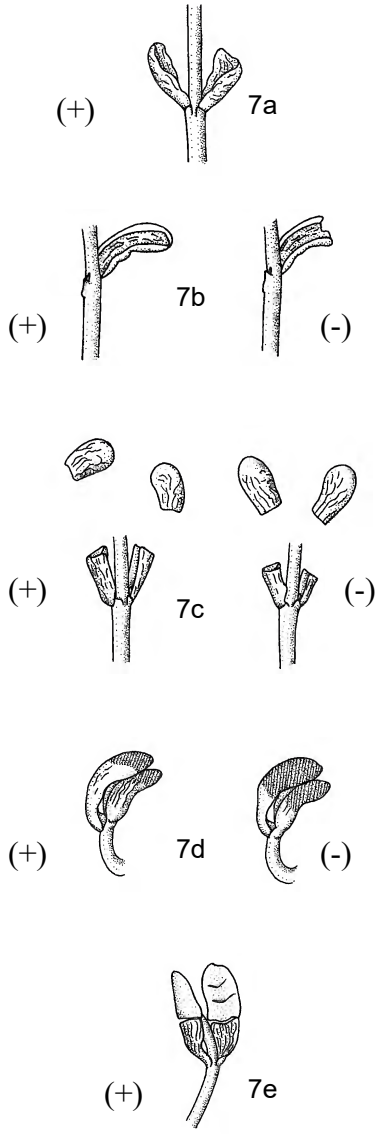


Fig. 7 Cotyledons, garden bean.



6a. Cotyledons missing but seedling vigorous.

6b. Cotyledons missing and seedling weak.

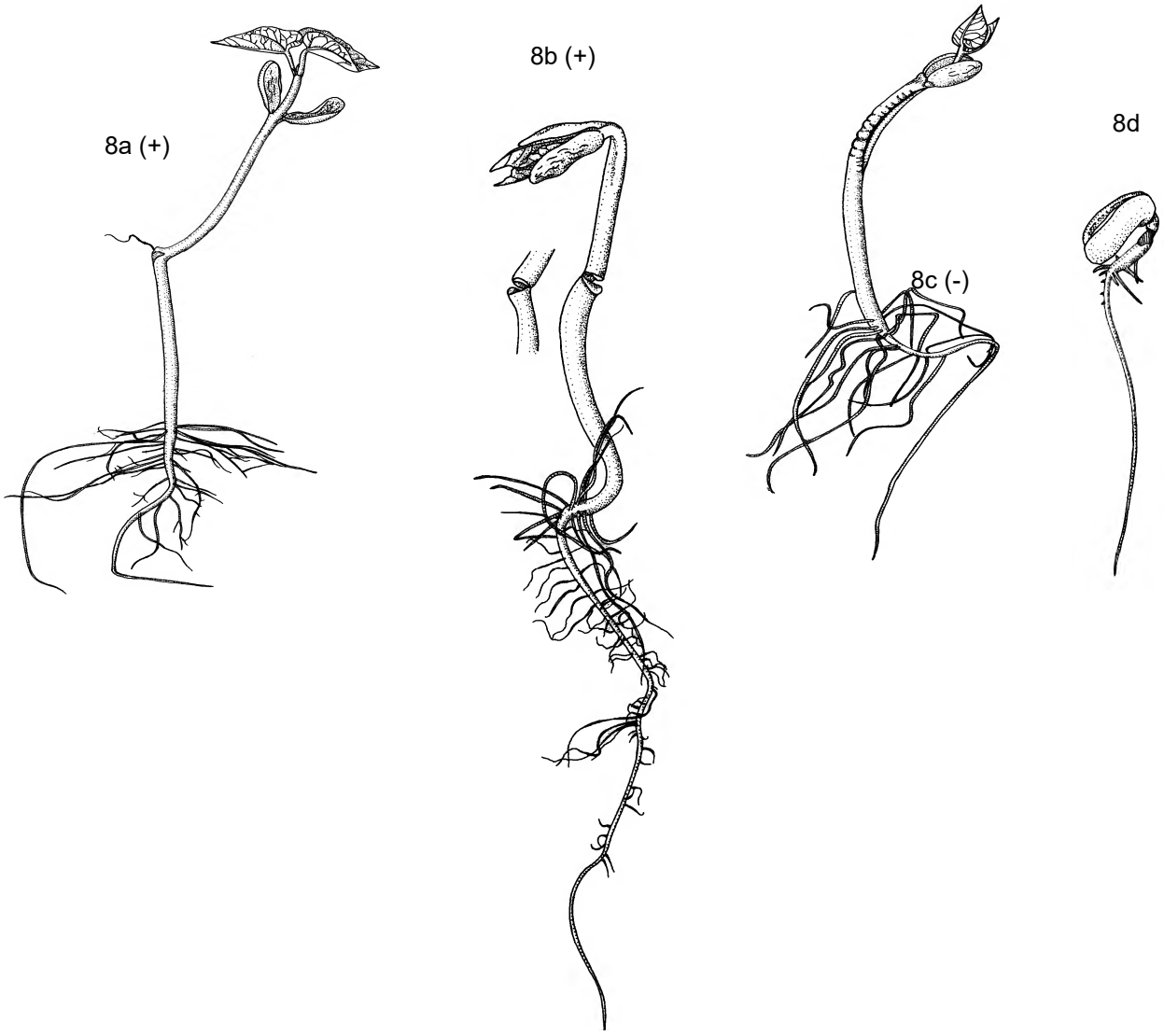
7a. Cotyledons shriveled, but intact.

7b. One cotyledon missing.

7c. Parts of both cotyledons missing.

7d. More than 50% of total cotyledon tissue decayed (this does not include firm, sound discolored tissue).

7e. Part of cotyledons non-functional but attached.



8a. Healed lesion (“knee”).

8b. Hypocotyl break due to towel test.

8c. Deep lesion.

8d. Hypocotyl collar rot (retest using calcium nitrate, see 4.7.3)

Fig. 8 Hypocotyl defects.

4.14.9 Fabaceae, Legume Family II - Soybean and Lupine

Glycine max, soybean
Lupinus spp., lupine, lupin (grain and forage)

General Description

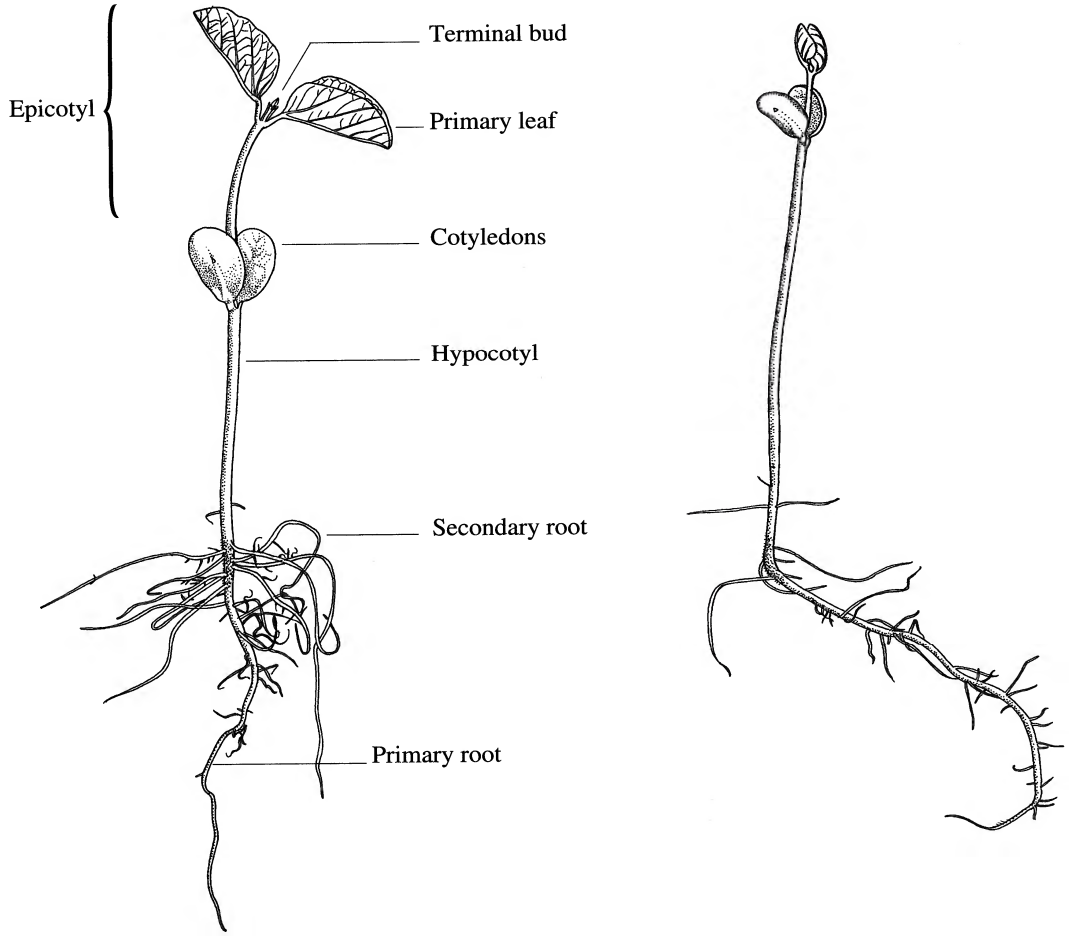
Seedling type: Epigeal dicot.

Food reserves: Cotyledons, which are large and fleshy; they expand, become photosynthetic and are usually persistent beyond the seedling stage.

Shoot system: The hypocotyl elongates and carries the cotyledons above the media surface. The primary leaves usually increase in size and there may be epicotyl elongation within the test period.

Root system: A long primary root with secondary roots.

1a. Sand test **Fig. 1 Soybean** **1b. Towel test**



Abnormal Seedling Description

Cotyledons:
- less than half of the original cotyledon tissue remaining attached.
- less than half of the original cotyledon tissue free of necrosis or decay.

Epicotyl:
- missing.
- less than one primary leaf.
- deep, open cracks.
- terminal bud damaged, missing or decayed (but see note 3).

Hypocotyl:
- deep open cracks extending into the conducting tissue (see note 6).
- malformed, such as markedly shortened, curled or thickened (see notes 1 and 4).

Root:
- none.
- weak, stubby or missing primary root with weak secondary or adventitious roots.

Seedling:
- one or more essential structures impaired as a result of decay from primary infection (see note 2).
- albino.

Notes:

1. Towels rolled too tightly or tight rubber bands may constrict growing seedlings causing them to be malformed. Tight rolls, often in combination with a mid-test watering, may cause hypocotyl cracking or splitting. The tissue around such breaks often appears swollen.

2. Secondary infection is common in towel and blotter tests. Some pathogens (*Fusarium, Phomopsis, Rhizoctonia*) can spread through the substrate and infect seedlings some distance away from the primary source. Seedlings with secondary infection are to be classified as normal. A retest in sand or soil-less organic growing media may be advisable.

3. If a few seedlings with a partial decay of the epicotyl are found, they may be classified as normal, provided the hypocotyl and root are normal. The epicotyl on such seedlings usually does not decay when grown in a fairly dry environment and is exposed to light. A retest, preferably in sand or soil-less organic growing media, will aid in interpretation of such seedlings.

4. Hypocotyl development is slow until the roots start functioning; caution must be exercised to ensure slow seedlings are not classified as abnormal. Similarly, epicotyls may remain undeveloped if the roots and hypocotyls are late in their development. A retest, preferably in sand or soil-less organic growing media, will aid in interpretation of such seedlings.

5. A preliminary count is prescribed (see Section 4.6.2 Table 5 for first count days), but caution must be exercised to avoid the possibility of misinterpreting small seedlings or causing damage.

6. Adventitious roots may occur at the site of any injury, particularly on the hypocotyl and near the base of the cotyledons. If the injury is healed over the seedling is to be classified as normal.

7. Large-seeded legumes are especially susceptible to threshing or combine damage. Seed which has been mechanically damaged may produce seedlings with damaged primary roots, hypocotyls or epicotyls, or broken or detached cotyledons. Bruised areas are usually

necrotic or decayed. Damage at the point of attachment of the cotyledons may be difficult to evaluate if seedlings are removed too early in the test period.

8. Low vigour or weak seedlings are weak, spindly and small. They may have other defects, such as damaged cotyledons or hypocotyl lesions. If there are only a few (1-3) weak seedlings in your test, classify them as abnormal. However, if all the seedlings in the sample appear weak, classify the normal “weak” seedlings as normal and make a note of the samples appearance in the remarks of your worksheet.

9. Some seeds germinate more slowly than others and will produce seedlings which appear smaller than average for the test. Provided sufficient growth has occurred to enable observation of all essential structures, there is no apparent damage, and they are not low vigour or weak seedlings, these should be classified as normal.

EPICOTYL

The epicotyl is the part of the axis above the cotyledons and is made up of a stem, two primary leaves and a terminal bud. Growth of the epicotyl follows growth of the primary root and hypocotyl and so in seedlings from late-germinating seeds, the epicotyl may be quite small. When judging the size of the leaves, they should be considered in relation to the size of the rest of the seedling.

Leaf Size

Leaf size is affected by the growth environment and/or the presence of damage. Since the seedling puts its energy into growth of the root and hypocotyl first (to ensure establishment and emergence of the seedling), small seedlings may have very small primary leaves. However, the size of the primary leaves may be an indication that there is damage to the epicotyl, at or near the base of the leaves. In such cases, assessment of the seedling must be based on the damage and not on the size of the primary leaves. The size of the primary leaf for any particular seedling should be estimated in relation to the size of that seeding; i.e. the “normal” size of a leaf for a small seedling will be less than that for a larger seedling. In estimating the size of the primary leaf, the leaf area, not the leaf length is to be considered.

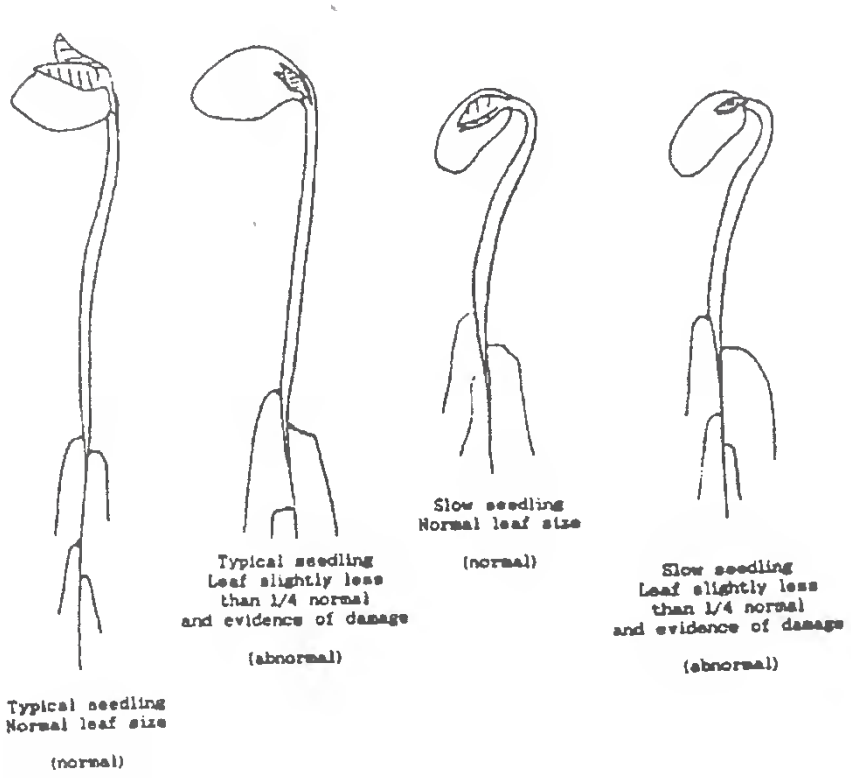


Fig. 2 Primary leaf size

Brown or Missing Leaves

A problem observed when tests are conducted under certain environmental conditions is that the primary leaves turn brown. In extreme cases these leaves shrivel and drop off, leaving a seedling with missing primary leaves. All seedlings with discoloured (brown and shrivelled) primary leaves, whether attached or detached (but still present) are to be classified as normal. However, should the detached primary leaves be missing, classify the seedling as abnormal.

If there is sufficient number of these seedlings to affect the grade of the lot, then a retest should be made using alternate test conditions. If there was no disease problem observed with the sample, a rolled towel test should give a better result with respect to the brown leaf condition. If there was

disease present, then a sand or soil test under higher light intensity and/or lower cabinet humidity should give better results.

Epicotyl "Bent-Over"

In soybean, the epicotyl is naturally bent over, resting between the cotyledons, until elongation pulls the primary leaves free. A bent-over epicotyl should be considered abnormal only if there is evidence of damage which would have caused the condition. Typically there is a lesion at the base of the epicotyl or at the point of attachment of the cotyledons. This damage results in unequal growth of tissues around the epicotyl, causing the epicotyl to bend. Sometimes the bending is accompanied by a failure of one of the primary leaves to develop at the same rate as the other. This is further evidence that there has been damage.

As a guideline, if the epicotyl is bent 90 degrees or more, as the result of damage, then it should be classified as abnormal. Damage occurring below the point of attachment of the cotyledons may cause a lesion of the hypocotyl which extends either into the epicotyl or into one of the cotyledons. A lesion which extends into the epicotyl may cause bending as described above, and such a seedling is abnormal. A lesion which extends up into the cotyledon may cause some bending of the upper hypocotyl, but usually does not cause bending of the epicotyl itself. This lesion should be judged as any other lesion, i.e. abnormal if deep, normal if not deep.

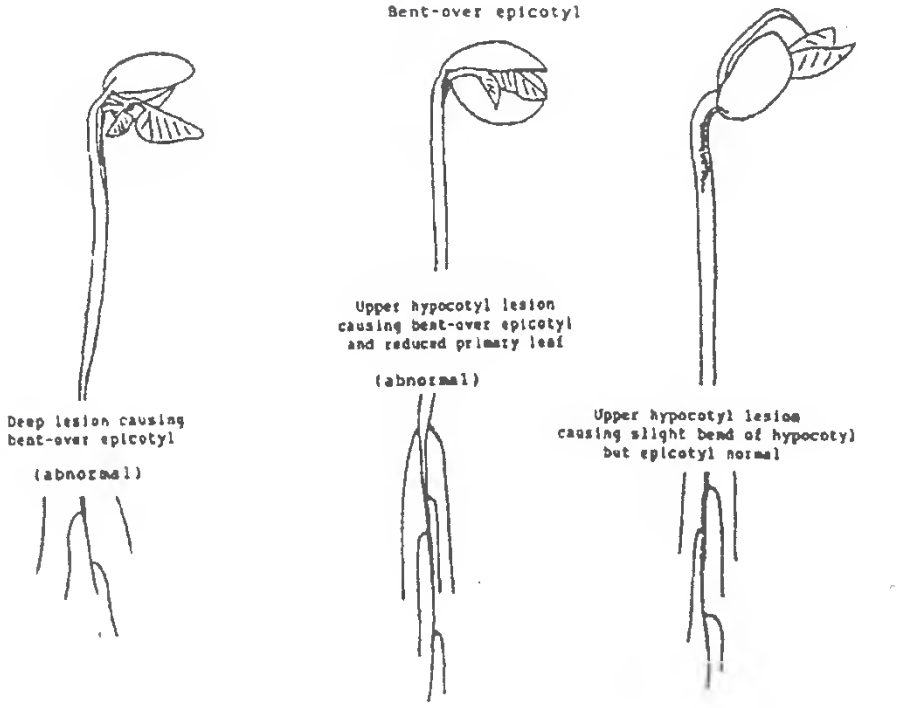


Fig. 3 Bent-over Epicotyl

HYPOCOTYL

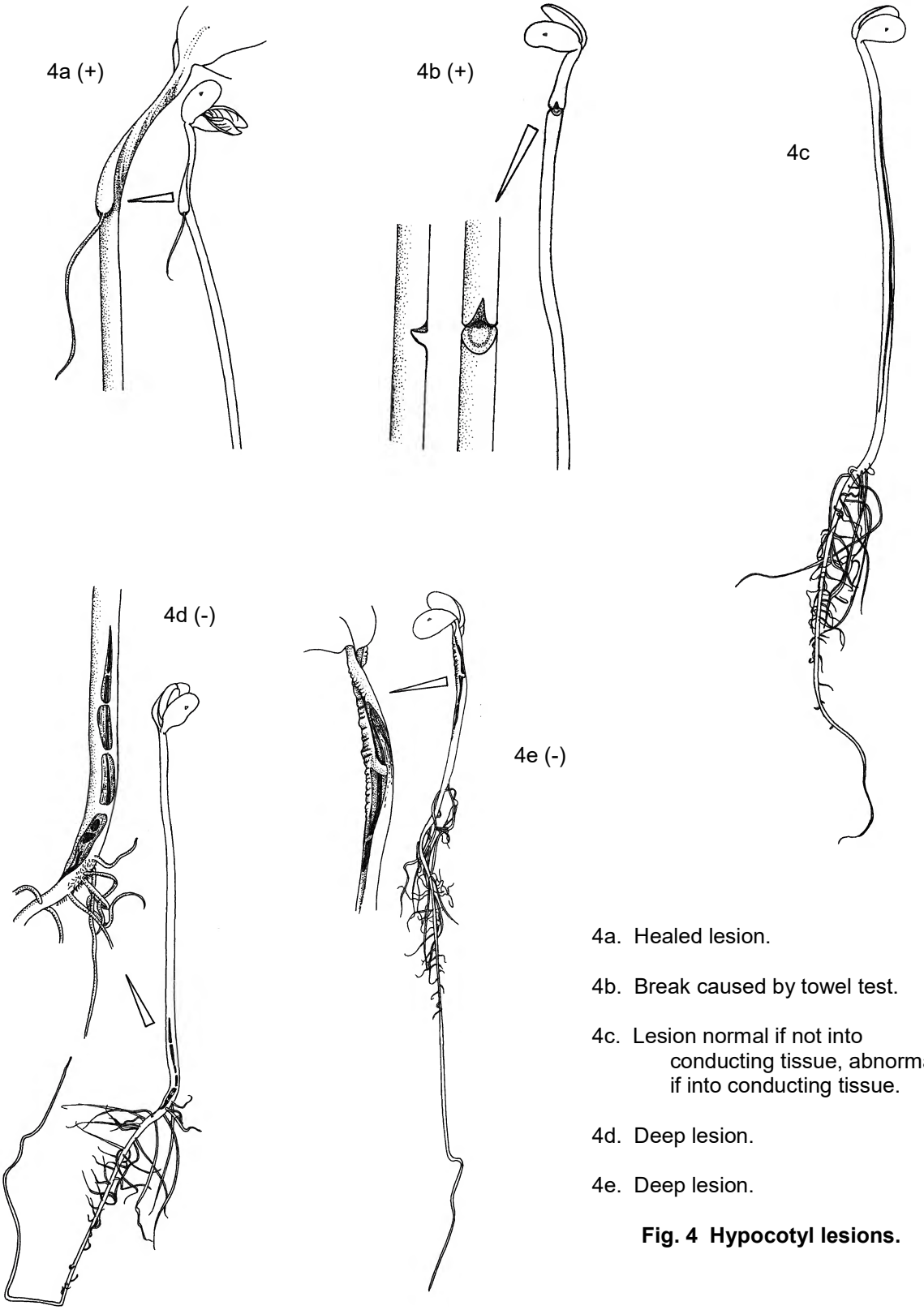
The hypocotyl develops from the part of the embryonic axis lying between the cotyledons and the radicle. Damage to this area of the axis will result in lesions in the seedling or short, thickened hypocotyls. Seedlings with severely shortened hypocotyls will have difficulty emerging in the field.

Lesions

Lesions are most often found in the hypocotyl, but may also be found in the epicotyl or root. Lesions are considered to be a serious defect because they interfere with the movement of water and nutrients through the affected area and increase susceptibility of the seedling to micro-organism (disease) attack.

Two factors which are to be considered when assessing the severity of lesions are the depth of the lesion and the degree of healing.

Seedlings with lesions extending into the conducting tissue are classified as abnormal. The analyst must therefore judge whether or not the lesion penetrates the outer layer of the central stele; the best way to do this is to break or cut the structure at the point where the lesion appears to be the deepest.



4a. Healed lesion.

4b. Break caused by towel test.

4c. Lesion normal if not into conducting tissue, abnormal if into conducting tissue.

4d. Deep lesion.

4e. Deep lesion.

Fig. 4 Hypocotyl lesions.

The root/shoot axis is made up of a central stele (or vascular cylinder), surrounded by the cortex and epidermis. The conducting tissue is contained in the outer layers of the stele, and serves to transport water and nutrients. The conducting tissue is not readily visible without magnification, but the analyst should be able to estimate its location. It is strongly recommended that analysts spend some time observing the structures of the seedling under the microscope. It is particularly useful to observe various types of lesions in cross section, in order to get a "feel" for the link between appearance and severity.

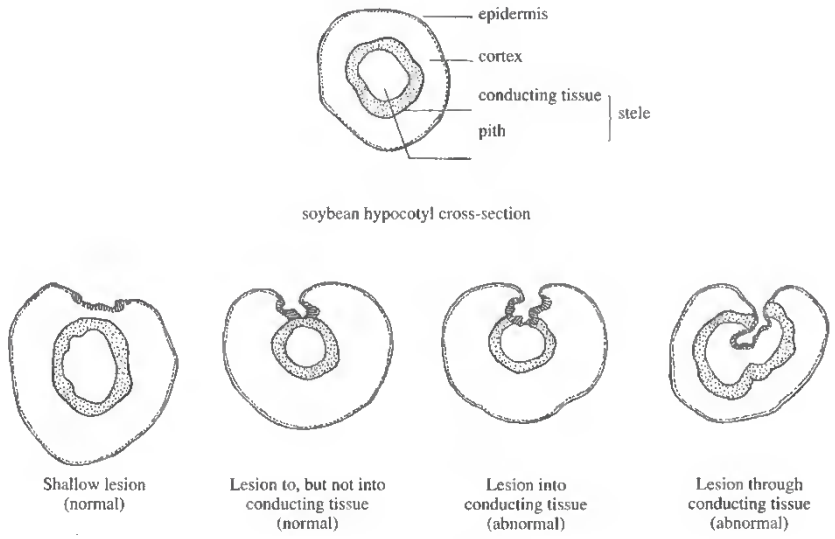


Fig. 5 Soybean hypocotyl cross-section (AOSA Seedling Evaluation Handbook)

In general, four levels of depth occur.

a. **Surface.** Only the epidermal layer is affected. The seedling often appears as having been "skinned", and may have brown striations running across the lesion. When the affected area is observed in cross-section, there is no significant indentation into the inner tissues. The hypocotyl has retained its normal rounded shape in the affected area. These seedlings are to be classified as normal

b. **Severe "Deep"**. These lesions go right through the vascular tissues, into or through the centre of the stele (into the pith), and are easily identified as being severe. These seedlings are to be classified as abnormal.

c. **Severe "Shallow".** These lesions have an appearance similar to the "skinned" or surface lesion, in that when cut, there may not be a significant indentation visible, and they may have brown striations running across the lesion. These lesions are typically broad and appear healed. They can be distinguished from a surface lesion by the "lumpy" appearance and feel of the unaffected side of the hypocotyl. The hypocotyl is also somewhat flattened in the area of the lesion. The lesion in these seedlings has removed a significant portion of the vascular tissue and is to be classified as abnormal

d. **Intermediate.** These are the most difficult to judge, since the depth in relation to the position of the central stele is not readily apparent. The seed analyst must cut or break the structure (ie. hypocotyl and/or epicotyl) at the most severe point of the lesion and make a judgement as to depth.

Healing

Lesions should be considered as healed if the gap caused by the lesion has been filled in by the production of cells from the surrounding tissues. This may result in swelling of the affected area and may appear as a "knee" such as is often seen in field or garden beans. Such seedlings are classified as normal. If there is a gap which has not completely filed in and there is still an opening into the hypocotyl, then the lesion should be judged as any other lesion, ie. abnormal if deep, normal if not deep.

Before the growth of seedling structures such as the hypocotyl begins, any damaged or dead tissue in the embryo often turns brown. As growth of the healthy tissue occurs the damaged or dead tissue is stretched and breaks apart. This tissue shows up as brown spots or striations along the length of the lesion. These brown areas should not be interpreted as evidence of “healing”.

Damaged Hypocotyl

This type of small seedling is usually the result of mechanical damage caused by harvesting and/or seed cleaning procedures. It is usually manifested in the seedlings as a missing primary root and significantly shortened hypocotyl, or as fractures at the base of the cotyledons with very little root development. These seedlings are to be classified as abnormal if they do not meet both of the following requirements:

1. The seedling’s hypocotyl length is equal to or greater than the length of one of its own cotyledons and,

2. There are sufficient secondary or adventitious roots.

ROOTS

The primary root develops from the radicle which forms the lower part of the embryonic axis. Depending on the degree of damage to the radicle, the primary root may vary in length from normal to completely missing, or it may have lesions or be diseased.

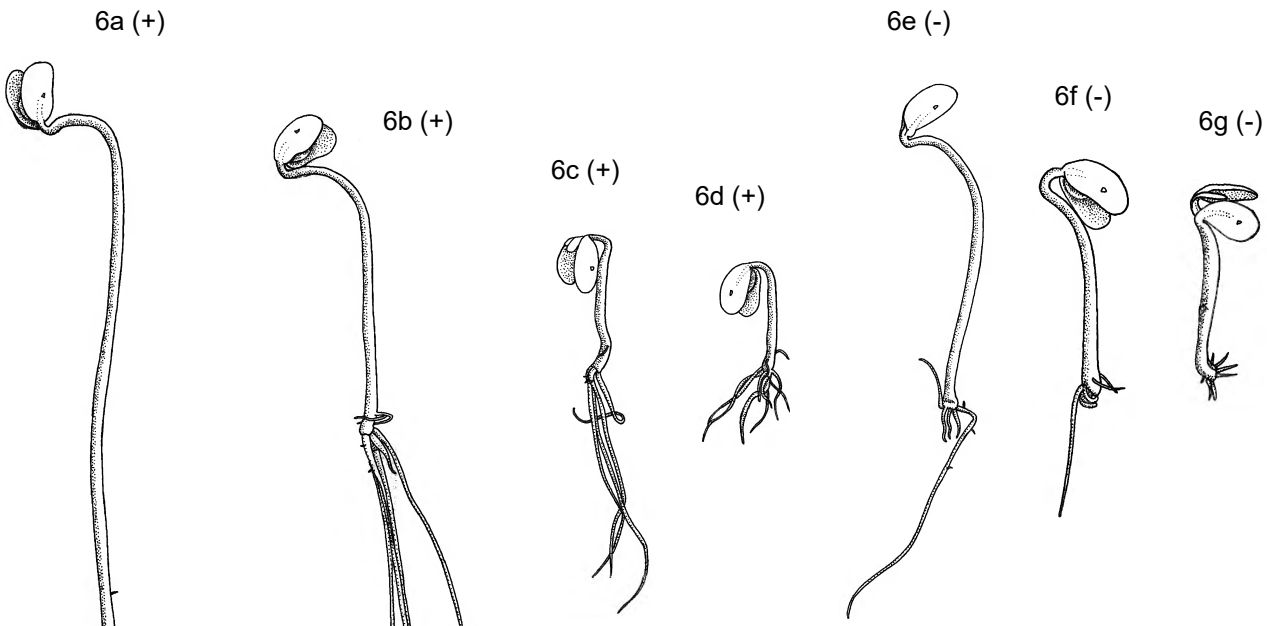
Adventious Roots

Adventitious roots may develop from the base of the hypocotyl and form the principal root system if the radicle has been completely destroyed. Such seedlings can be classified as normal provided these adventitious roots are strong and the hypocotyl has sufficient length.

Root Lesions

Lesions in the primary root, whether on the surface of the root or displayed as a split root, are not to be assessed as long as there is sufficient healthy secondary root development between the lesion and the hypocotyl. However, if the lesion or split in the primary root is large enough to breach the conducting tissue in the hypocotyl, then the seedling is to be classified abnormal.

Fig. 6 Root defects.



6a. Intact seedling.

6b.
6c.
6d.
6e.
Primary root missing, weak or stubby, sufficient secondary roots.

6f.
6g.
Primary root missing, weak or stubby, insufficient secondary roots.

DISEASED SEEDLINGS

Infection is the entrance and spread of disease organisms in living material (eg. seedling structures) often causing disease symptoms and decay. Two modes of infection are observed in seed testing:

Primary Infection:the disease organism is present and active in the seed and/or seedling itself.

Secondary Infection: the disease organism has spread from adjacent seeds or seedlings.

Unless there is clear evidence that the infection is secondary (ie. the analyst can directly observe that the source of infection is a neighbouring seed or seedling), it must be assumed that the infection is primary. If there is an excessive amount of secondary infection, such that it would be difficult to identify primary infection, then the sample should be retested using an alternate germination procedure (such as increasing the seed spacing).

Seedlings with primary infection are to be assessed as follows:

Cotyledons

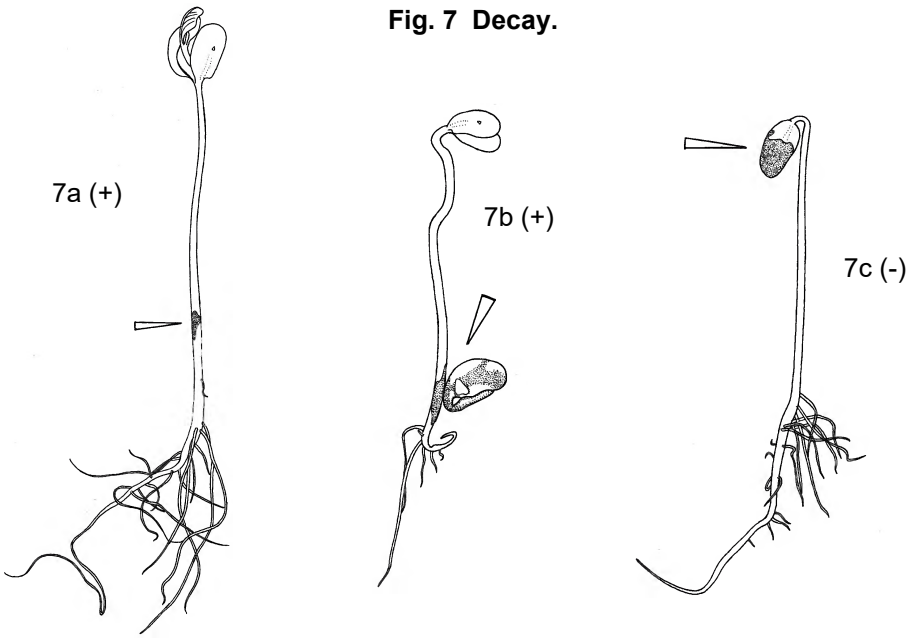
Follow the 50% rule, ie. If more than 50% of the cotyledonary tissue is infected, the seedling is classified as abnormal. It may be necessary to break open the cotyledons to determine the degree of penetration of infection. Remember to assess the total volume of the cotyledons not just the surface.

Epicotyl, Hypocotyl, Roots

Once it has been determined that the infection is primary, the analyst must judge whether the infection has caused the structure to be impaired to the extent that it is unable to function. If the infection is superficial, ie. it does not extend deep enough to affect the central stele the seedling is classified as normal. If the infection penetrates deeply into the central stele (ie. further development of the structure has been impaired) then the seedling is classified as abnormal. Therefore, when assessing diseased epicotyls and hypocotyls, the same criteria used for depth of lesions should be used for assessing the degree of penetration of infection.

Disease on the primary root is not to be assessed as long as there is sufficient healthy secondary root development between the decay and the hypocotyl. If there are not sufficient healthy roots, then the disease is to be assessed as described above for the epicotyl and hypocotyl.

Fig. 7 Decay.



7a. Decay on surface.
7b. Secondary infection from another seed or seed coat.
7c. More than 50% of total cotyledon tissue decayed (this does not include firm, sound, discolored tissue).

4.14.10 Fabaceae, Legume Family IV - Large-Seeded Hypogeal

Cicer arietinum, chickpea
Lens culinaris, lentil
Phaseolus coccineus, runner bean
Pisum sativum, pea (field or garden)
Vicia faba, horse broad, tick or faba bean
Vicia spp., vetch

General Description

Seedling type: Hypogeal dicot.

Food reserves: Cotyledons which are large and fleshy, and remain enclosed within the seed coat beneath the media surface. They are usually not photosynthetic.

Shoot system: The epicotyl elongates and carries the terminal bud and primary leaves above the media surface. The stem bears one or more scale leaves and, prior to emergence, is arched near the apex, causing the terminal bud to be pulled through the medial; after emergence, the stem straightens. For practical purposes the hypocotyl is not discernible and is not an evaluation factor. There are buds in the axils of each cotyledon and scale leaf but these usually remain dormant unless the terminal bud is seriously damaged. In this case, one or more auxiliary buds will start to develop, forming a secondary epicotyl.

Root system: A long primary root with secondary roots.

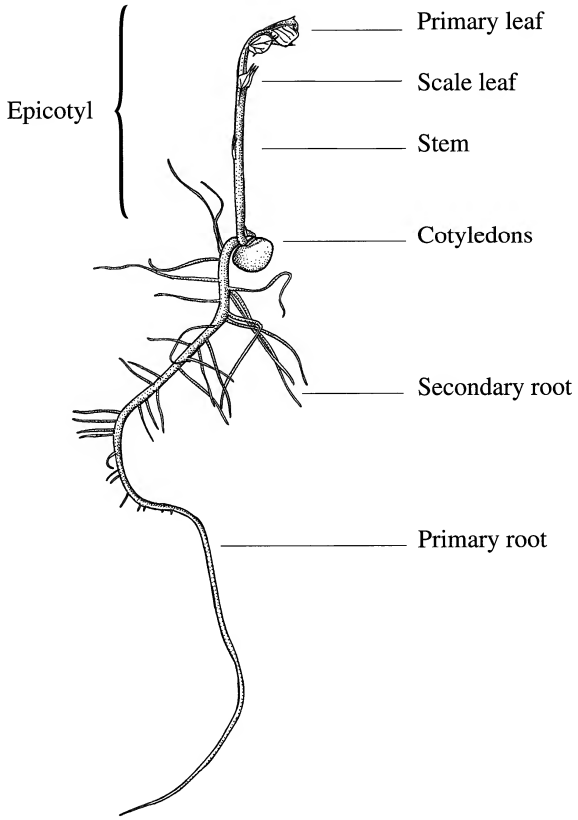


Fig. 1 Pea.

Abnormal Seedling Description

Cotyledons:

- less than half of the original cotyledon tissue remaining attached (see notes 4 and 5).
- less than half of the original cotyledon tissue free of necrosis or decay.

Epicotyl:

- missing.
- less than one primary leaf.
- malformed stem such as markedly shortened, curled, or thickened.
- severely damaged (e.g. terminal bud missing or damaged) with only a weak secondary epicotyl developing from the axil of a cotyledon or scale leaf.
- two weak epicotyls.
- deep, open cracks extending into the conducting tissue.

Root:

- none.
- weak, stubby or missing primary root with weak secondary roots.

Seedling:

- one or more essential structures impaired as a result of decay from primary infection.
- albino.

Notes:

1. There is a greater likelihood of hard seed expression when the substrate does not provide adequate moisture to the seeds throughout the test period.

2. Insufficient moisture will result in apparently disproportionate elongation of the primary root and slow development of the epicotyl.

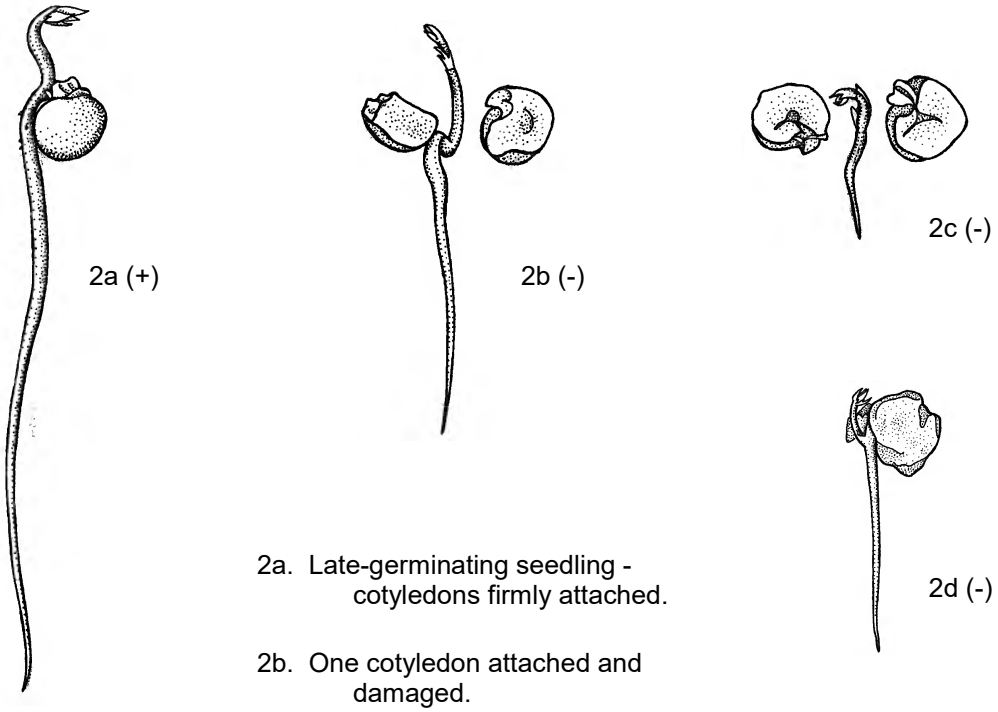
3. Manganese deficiency at the time of seed development may cause a condition known as "marsh spot", characterized by a discoloured brown indentation in the center of the inner surfaces of the cotyledons. Seedlings with this condition are considered normal, provided they are otherwise normal. If the condition causes difficulty in evaluation, then the sample must be retested in soil-less organic growing media.

4. Weevil infestation may prevent the development of a normal seedling. Sometimes the cotyledons have been devoured to the extent that no food supply is left for the developing seedling. Such injury can be easily detected by examining the cotyledons.

5. Large-seeded legumes are especially susceptible to threshing or combine damage. Seed which has been mechanically damaged may produce seedlings with damaged primary roots, hypocotyls or epicotyls, or broken or detached cotyledons. Bruised areas are usually necrotic or decayed. Damage at the point of attachment of the cotyledons may be difficult to evaluate if seedlings are removed too early in the test period.

6. The percentage of hard seeds must be determined at the end of the test period for Hairy Vetch and Common Vetch of Grade Table II.1. Swollen seeds which fail to germinate by the end of the test must be allowed additional days as provided in Section 4.9.3 and 4.10.7.

Fig. 2 Cotyledon defects.

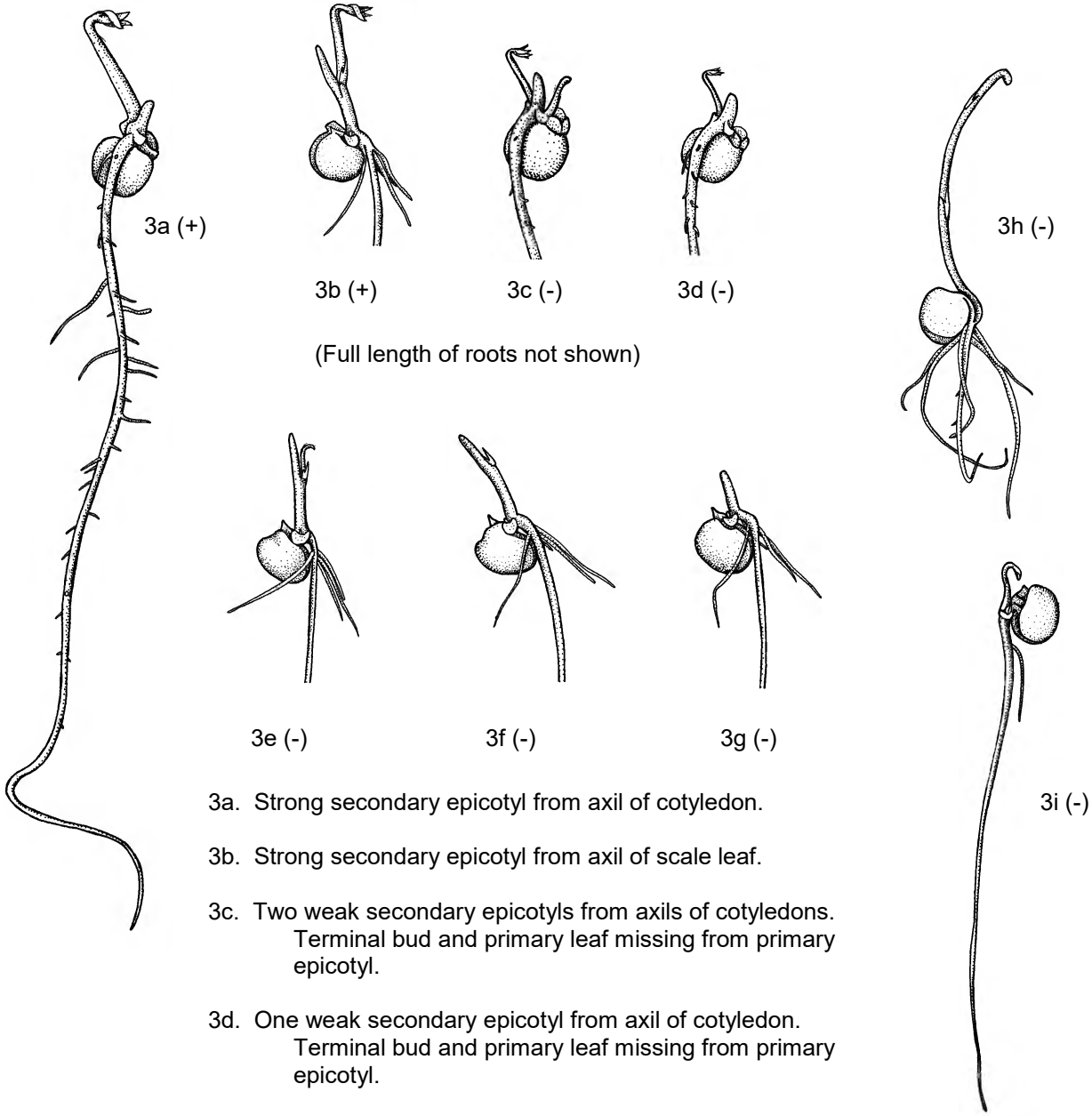


2a. Late-germinating seedling - cotyledons firmly attached.

2b. One cotyledon attached and damaged.

2c. Both cotyledons detached.

2d. Cotyledons attached, but more than half decayed.



3a. Strong secondary epicotyl from axil of cotyledon.

3b. Strong secondary epicotyl from axil of scale leaf.

3c. Two weak secondary epicotyls from axils of cotyledons. Terminal bud and primary leaf missing from primary epicotyl.

3d. One weak secondary epicotyl from axil of cotyledon. Terminal bud and primary leaf missing from primary epicotyl.

3e. One weak secondary epicotyl from axil of scale leaf.

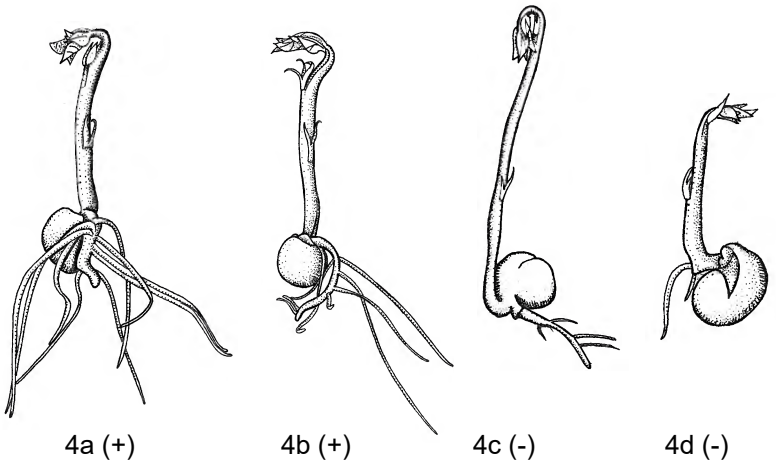
3f. Terminal bud and primary leaf missing.

3g. Terminal bud and primary leaf missing.

3h. Terminal bud and primary leaf missing.

3i. Weak epicotyl. One or both cotyledons detached.

Fig. 3 Epicotyl defects.



4a (+) 4b (+) 4c (-) 4d (-)

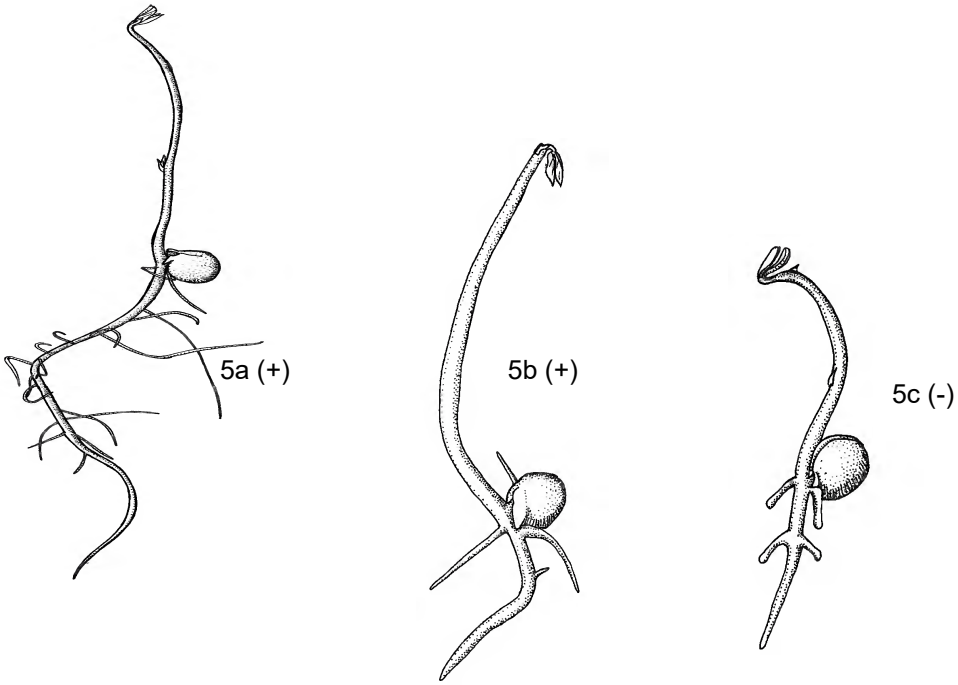
4a. Stubby primary root, sufficient secondary roots.

4b. Stubby primary root, sufficient secondary roots.

4c. Stubby primary root, insufficient secondary roots.

4d. Insufficient roots.

Fig. 4 Root defects – Pea.



5a. Intact lentil seedling.

5b. Stubby primary root with secondary roots developing.

5c. Stubby primary root with stubby secondary roots.

Fig. 5 Root defects – Lentil.

4.14.11 Fabaceae, Legume Family V - Small-Seeded

Anthyllis vulneraria, kidneyvetch
Astragalus cicer, cicer milkvetch
Securigera varia, crownvetch
Lespedeza cuneata, Lespedeza (sericea or Chinese)
Kummerowia spp., lespedeza (common, Kobe or Korean)
Lotus corniculatus, trefoil
Medicago spp., alfalfa, black medick
Melilotus spp., sweet clover
Onobrychis viciifolia, sainfoin
Trifolium spp., clover

General Description

Seedling type: Epigeal dicot.

Food reserves: Cotyledons, which are small and fleshy; they expand, and become photosynthetic.

Shoot system: The hypocotyl elongates and carries the cotyledons above the media surface. The epicotyl usually does not show any development within the test period.

Root system: A long tapering primary root, usually with roots hairs. Most of the included species do not normally develop secondary roots within the test period.

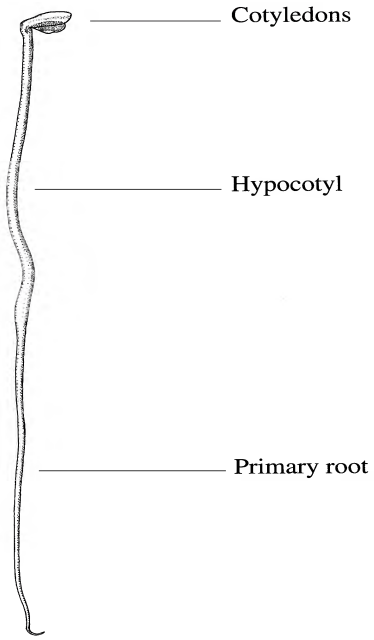


Fig. 1 Alfalfa.

Abnormal Seedling Description

Cotyledons:
- less than half of the original cotyledon tissue remaining attached (see note 2).
- less than half of the original cotyledon tissue free of necrosis or decay.

Epicotyl:
- missing (may be assumed to be present if cotyledons are intact).

Hypocotyl:
- deep open cracks extending into the conducting tissue.
- malformed, such as markedly shortened, curled or thickened.
- watery.

Root:
- none.
- primary root stubby (for sweet clover and crownvetch, or for roots bound by the seed coat see note 1).
- split extending into the hypocotyl.

Seedling:
- one or more essential structures impaired as a result of decay from primary infection.
- albino.

Notes:

1. Stubby roots when germinated on artificial media:
   - Sweet clover -- the roots of sweet clover may be stubby due to the presence of coumarin in the seed. Since this condition usually does not occur in media, such seedlings are to be classified as normal.
   - Bound by coat -- roots may appear stubby as a result of being bound by the seed coat. Such seedlings are to be classified as normal.
   - Crownvetch -- produces phytotoxic effects similar to sweet clover.

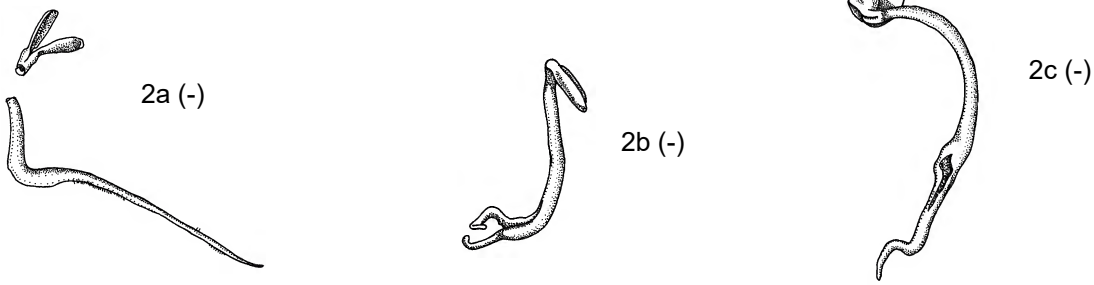
2. Breaks at the point of attachment of the cotyledons to the hypocotyl are common in seeds which have been mechanically damaged. It is important that seedlings not be removed during preliminary counts unless development is sufficient to allow the condition of the cotyledons to be determined. If the point of attachment of the cotyledons cannot be seen at the end of the test, the seed coat must be peeled back to determine whether a break has occurred.

3. Mechanical breakage of the seed may result in only vestiges of seedlings with swollen cotyledons and broken, slightly enlarged hypocotyls or radicles. Insect damage may also cause lack of seedling growth.

4. Seedlings of sainfoin which have been "strangled" by growing through the netting of the pod but which are otherwise normal are to be classified as normal.

5. The percentage of hard seeds must be determined at the end of the test period for all genera in this group. Swollen seeds which fail to germinate by the end of the test must be allowed additional days as provided in Section 4.9.3 and 4.10.7. Swollen seeds are an indication of dormancy and can be induced by incorrect temperatures.

Fig. 2 Hypocotyl defects.



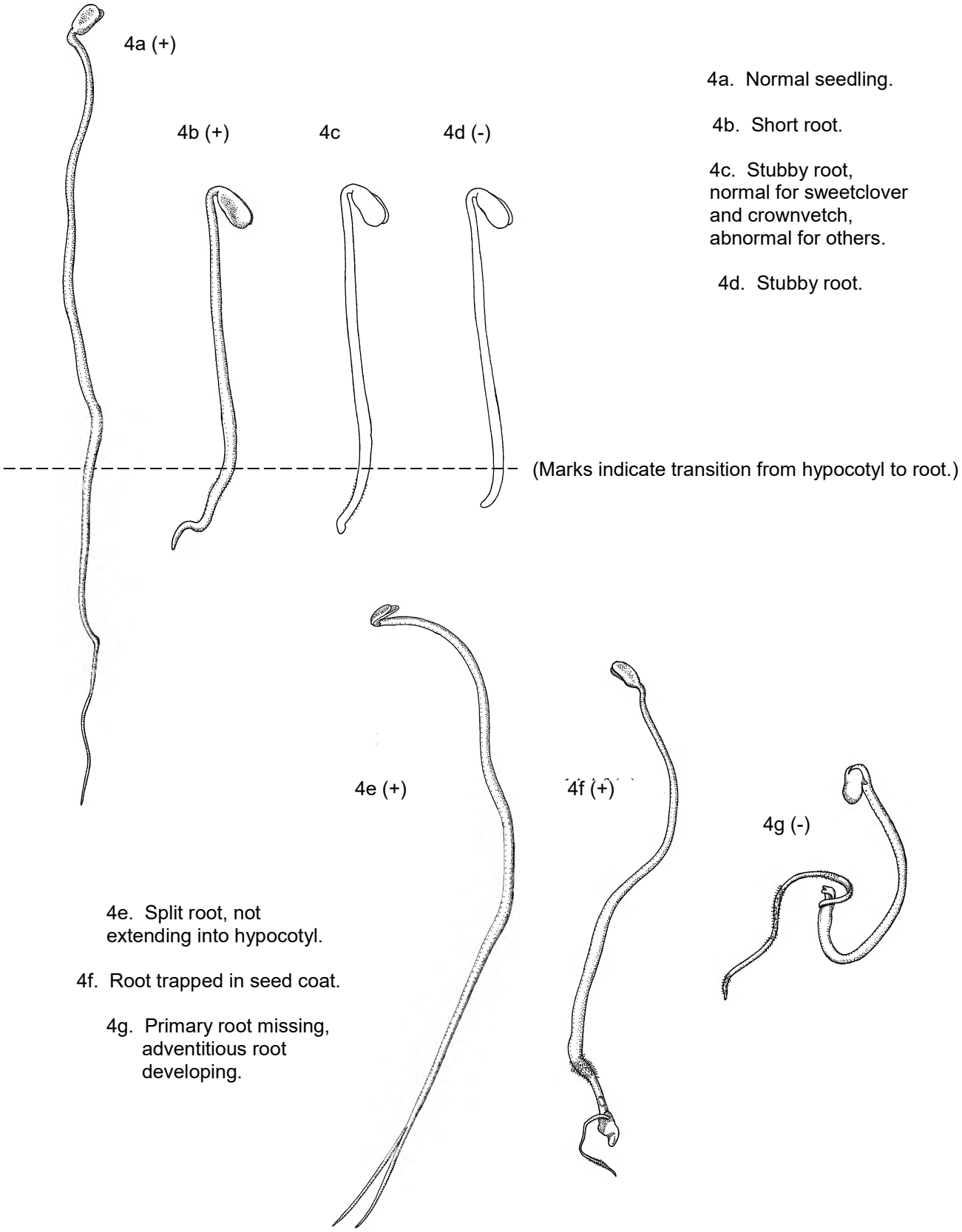
2a. Broken hypocotyl.
2b. Split root and hypocotyl.
2c. Deep lesion of hypocotyl.

3a (+) 3b 3c (-)

Fig. 3 Small seedlings.

3a. Late-germinating seedling
3b. Seedling too small to evaluate
3c. Swollen hypocotyl, stubby root.

Fig. 4 Root Defects



4a. Normal seedling.

4b. Short root.

4c. Stubby root, normal for sweetclover and crownvetch, abnormal for others.

4d. Stubby root.

4e. Split root, not extending into hypocotyl.

4f. Root trapped in seed coat.

4g. Primary root missing, adventitious root developing.

4.14.12 Liliaceae, Lily Family I - Asparagus

Asparagus officinalis, asparagus

General Description

Seedling type: Hypogeal monocot.

Food reserves: Endosperm which is hard, semi-transparent and non-starchy; minor reserves in the cotyledon. The endosperm surrounds the entire embryo.

Cotyledon: A single cylindrical cotyledon; following germination all but the basal end remains embedded in the endosperm to absorb nutrients.

Shoot system: The epicotyl elongates and carries the terminal bud and primary leaves above the media surface. The epicotyl may bear several small scale leaves. A short hypocotyl is barely distinguishable joining the root to the basal end of the cotyledon, which emerges from the seed.

Root system: A long slender primary root.

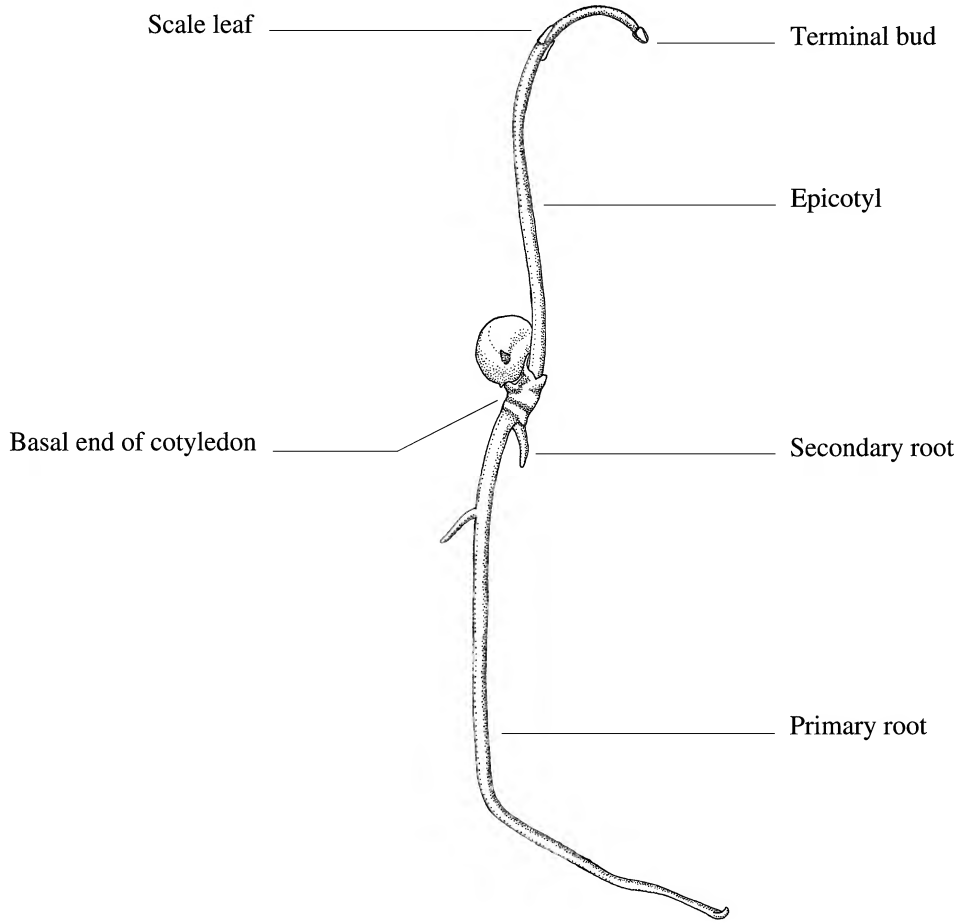


Fig. 1 Asparagus.

Abnormal Seedling Description

Cotyledon:
- detached from seedling.

Epicotyl:
- missing.
- terminal bud missing or damaged.
- deep, open cracks.
- malformed, such as markedly shortened, curled, or thickened.
- spindly.
- watery.
- (see also note 1).

Hypocotyl:
- not evaluated.

Root:
- no primary root.
- stubby primary root, with weak secondary roots

Seedling:
- one or more essential structures impaired as a result of decay from primary infection.
- albino.

Notes:

1. Several epicotyls may arise simultaneously and may be considered normal if at least one appears to be vigorous and has a terminal growing point.

2. Some seeds do not contain an embryo.

2a (-)

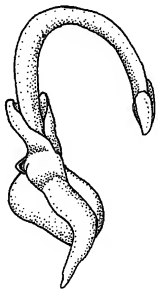


Fig. 2 Root defect.

2a. Stubby primary root.

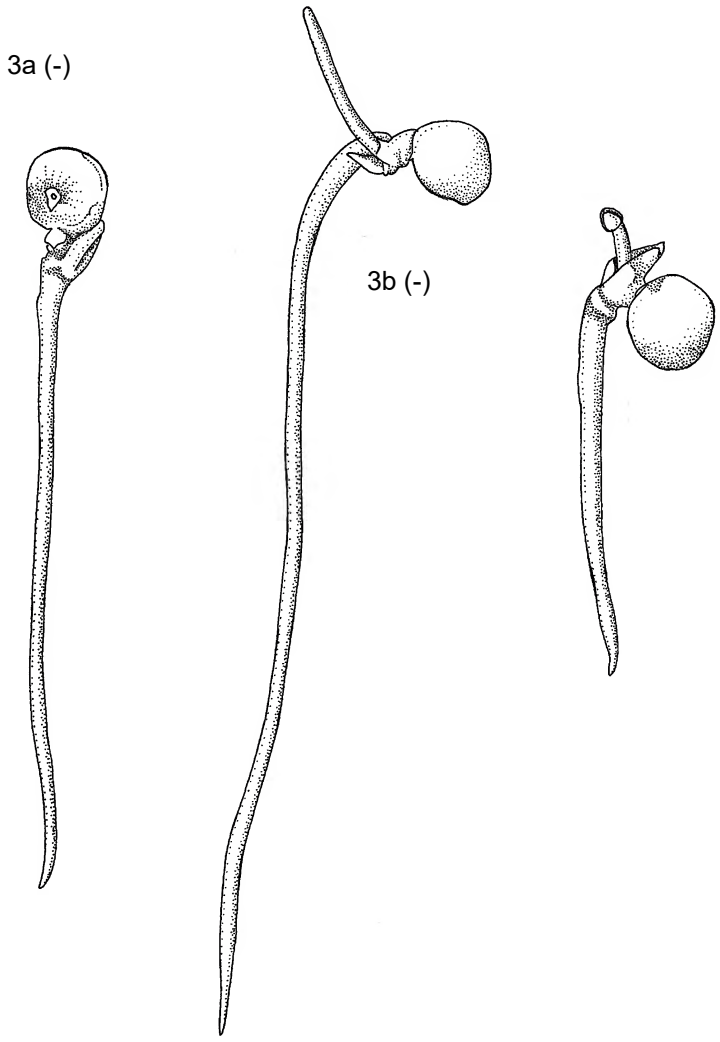


Fig. 3 Epicotyl defects.

3a. Epicotyl missing.

3b. Terminal bud missing.

3c. Epicotyl markedly shortened.

4.14.13 Liliaceae, Lily Family II - Onion, Leek and Chives

Allium cepa, onion
Allium porrum, leek
Allium schoenoprasum, chives

General Description

Seedling type: Epigeal monocot.

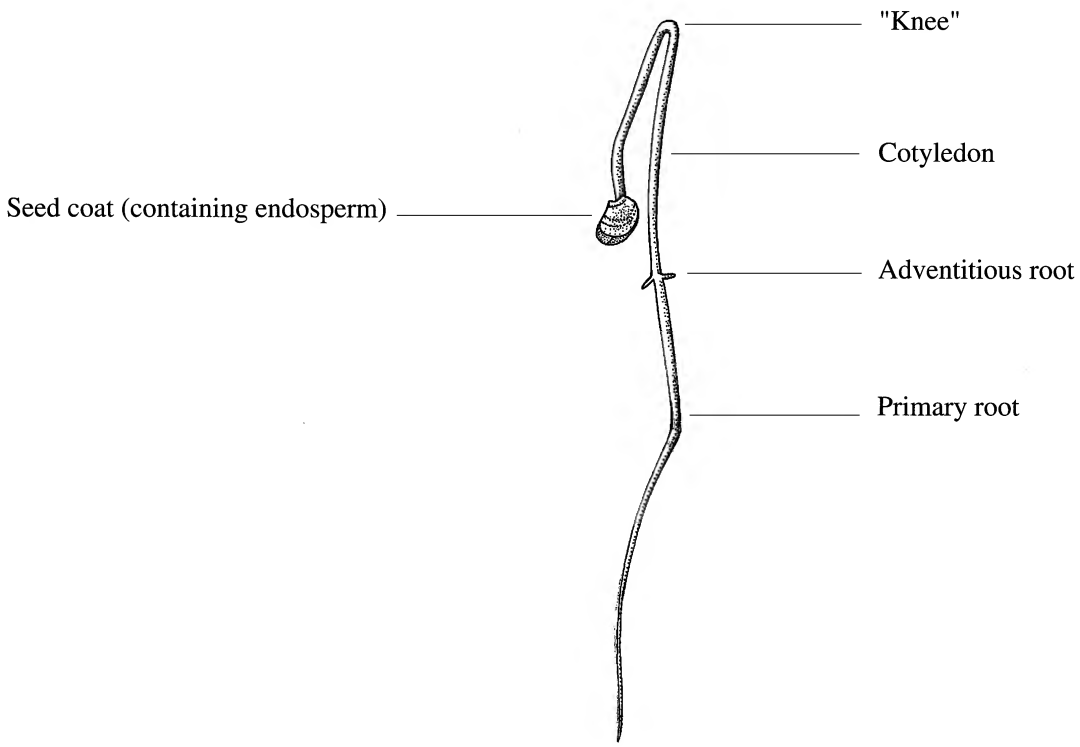
Food reserves: Endosperm which is hard, semi-transparent and non-starchy; minor reserves in the cotyledon.

Cotyledon: A single cylindrical cotyledon; following germination the tip remains embedded in the endosperm to absorb nutrients.

Shoot system: The cotyledon emerges with the seed coat and endosperm attached to the tip. A sharp bend known as the "knee" forms; continued elongation of the cotyledon on each side of this knee pushes it above the media surface. The cotyledon tip is pulled from the media and straightens except for a slight kink which remains at the site of the knee. The first foliage leaf emerges through a slit near the base of the cotyledon, but this does not usually occur during the test period. The hypocotyl is a very short transitional zone between the primary root and the cotyledon.

Root system: A long slender primary root with adventitious roots developing from the hypocotyl. The primary root does not develop secondary roots.

Fig. 1 Onion



Abnormal Seedling Description

Cotyledon:
- short and thick.
- without a definite bend or "knee".
- spindly or watery.

Epicotyl:
- not observed during the test period.

Hypocotyl:
- not evaluated.

Root:
- no primary root.
- short, weak or stubby primary root.

Seedling:
- one or more essential structures impaired as a result of decay from primary infection.
- albino.

Notes:

1. Excess moisture may cause a delay in germination causing some seed lots to appear dormant.

2. Blotter or towel tests of onion are commonly overcome with fungus. To reduce this problem on a retest, seeds must be spaced farther apart.

2a. Slight “knee” visible.

2b. No “knee” visible.

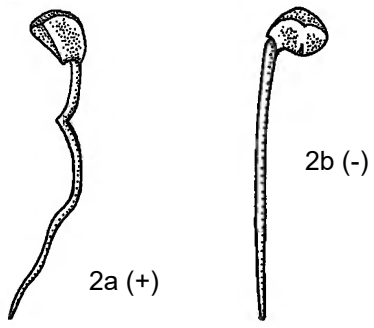


Fig. 2 Cotyledon “knee”.

3a. Slightly stubby root.

3b. Stubby root, with adventitious roots started.

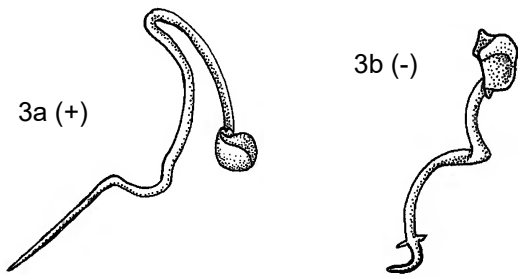


Fig. 3 Root defects.

4.14.14 Linaceae, Flax Family

Linum usitatissimum, flax

General Description

Seedling type: Epigeal dicot.

Food reserves: Cotyledons which expand and become photosynthetic. They persist for about one month following germination.

Shoot system: The hypocotyl elongates carrying the cotyledons above the media surface. The epicotyl usually does not show any development within the test period.

Root system: A primary root, with secondary roots usually developing within the test period.

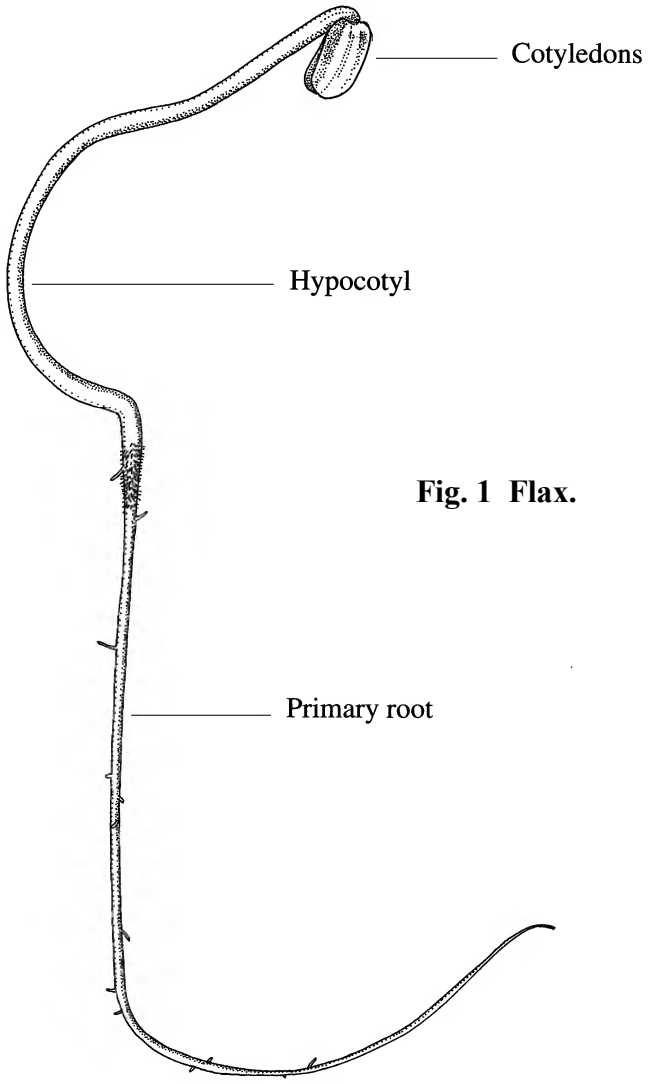


Fig. 1 Flax.

Abnormal Seedling Description

Cotyledons:
- less than half of the original cotyledon tissue remaining attached.
- less than half of the original cotyledon tissue free of necrosis or decay.

Epicotyl:
- missing (may be assumed to be present if cotyledons are intact).

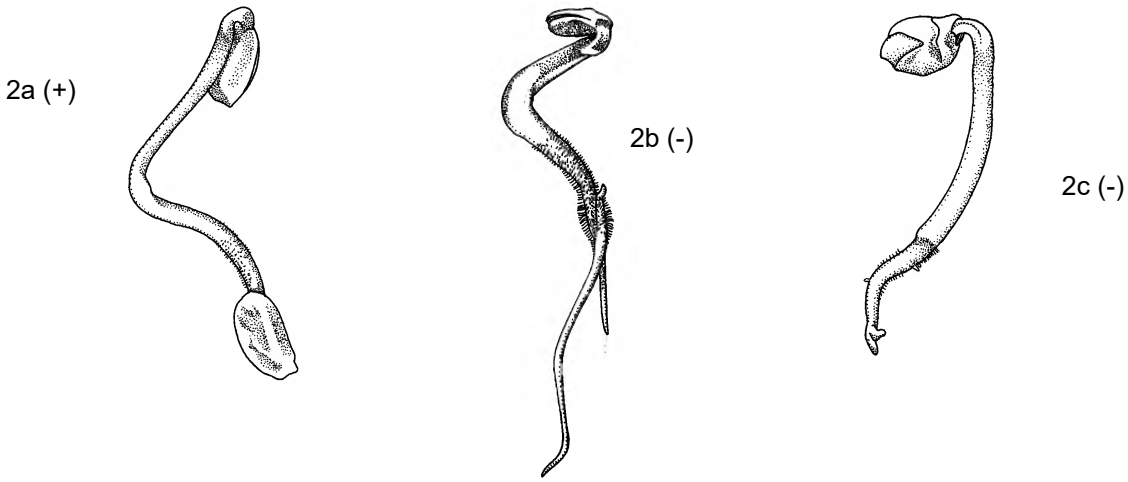
Hypocotyl:
- deep open cracks extending into the conducting tissue.
- malformed, such as markedly shortened, curled or thickened.

Root:
- none.
- weak, stubby or missing primary root with weak secondary roots.

Seedling:
- one or more essential structures impaired as a result of decay from primary infection.
- albino.

Notes:

1. Due to the mucilaginous nature of the seed coat, seedlings germinated on blotters may adhere to the blotter and appear to be negatively geotropic.



2a. Root trapped in seed coat.

2b. Deep lesion in root.

2c. Stubby root.

Fig. 2 Root defects.

Fig. 3 Hypocotyl defects.

3a. Deep lesion, hypocotyl twisted.

3b. Seed coat trapped, causing hypocotyl damage and decay.

3c. Thickened grainy hypocotyl.

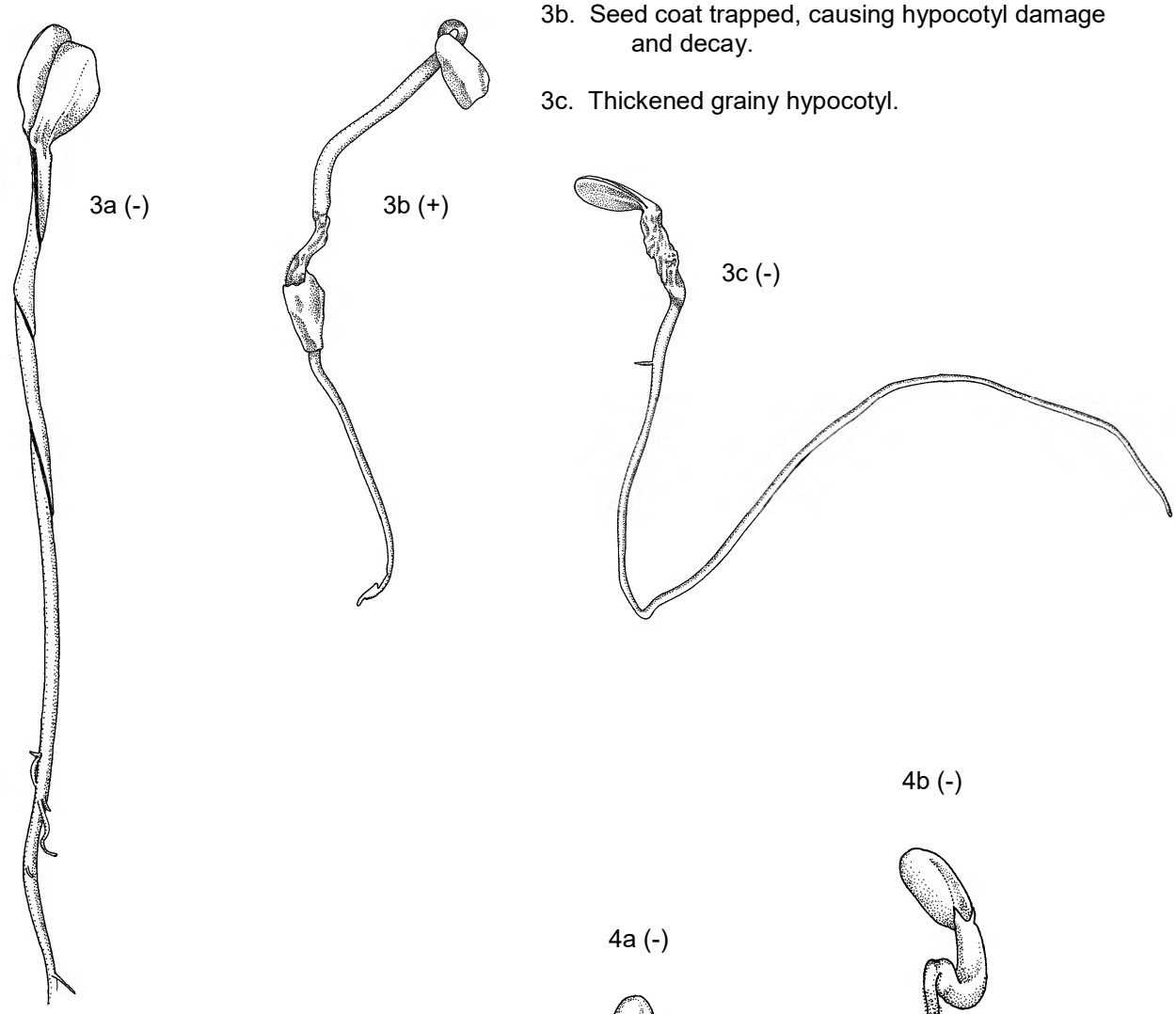
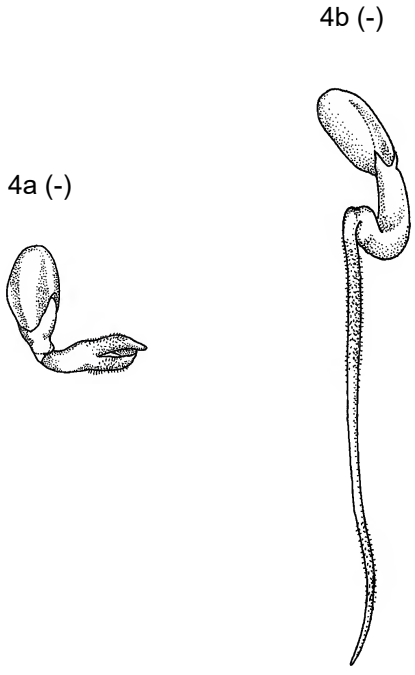


Fig. 4 Small seedlings.

4a. Short, thickened hypocotyl and root.

4b. Short, thickened hypocotyl.



4.14.15 Malvaceae, Mallow Family

Abelmoschus esculentus, okra

General Description

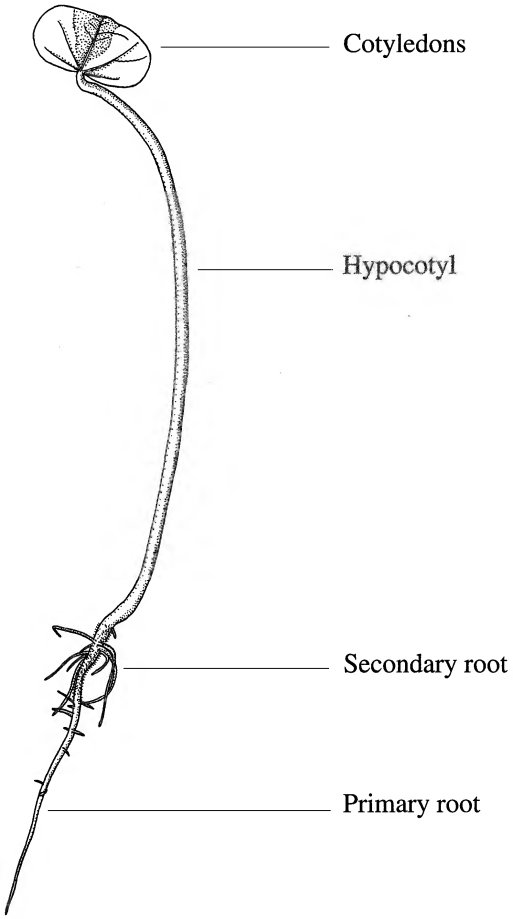
Seedling type: Epigeal dicot.

Food reserves: Cotyledons, which are much convoluted in the seed; they expand and become thin, leaf-like and photosynthetic.

Shoot system: The hypocotyl elongates carrying the cotyledons above the media surface. The epicotyl usually does not show any development within the test period.

Root system: A primary root, with secondary roots usually developing within the test period.

Fig. 1



Abnormal Seedling Description

Cotyledons:
- less than half of the original cotyledon tissue remaining attached.
- less than half of the original cotyledon tissue free of necrosis or decay.

Epicotyl:
- missing (may be assumed to be present if cotyledons are intact).

Hypocotyl:
- deep open cracks or grainy lesions extending into the conducting tissue.
- malformed, such as markedly shortened, curled or thickened.

Root:
- none.
- weak, stubby or missing primary root with weak secondary or adventitious roots.

Seedling:
- one or more essential structures impaired as a result of decay from primary infection.
- albino.

Notes:

None

2a. Intact seedling.

2b. Primary root missing, sufficient secondary roots.

2c. Primary root missing, insufficient secondary roots.

2d. Deep hypocotyl lesion.

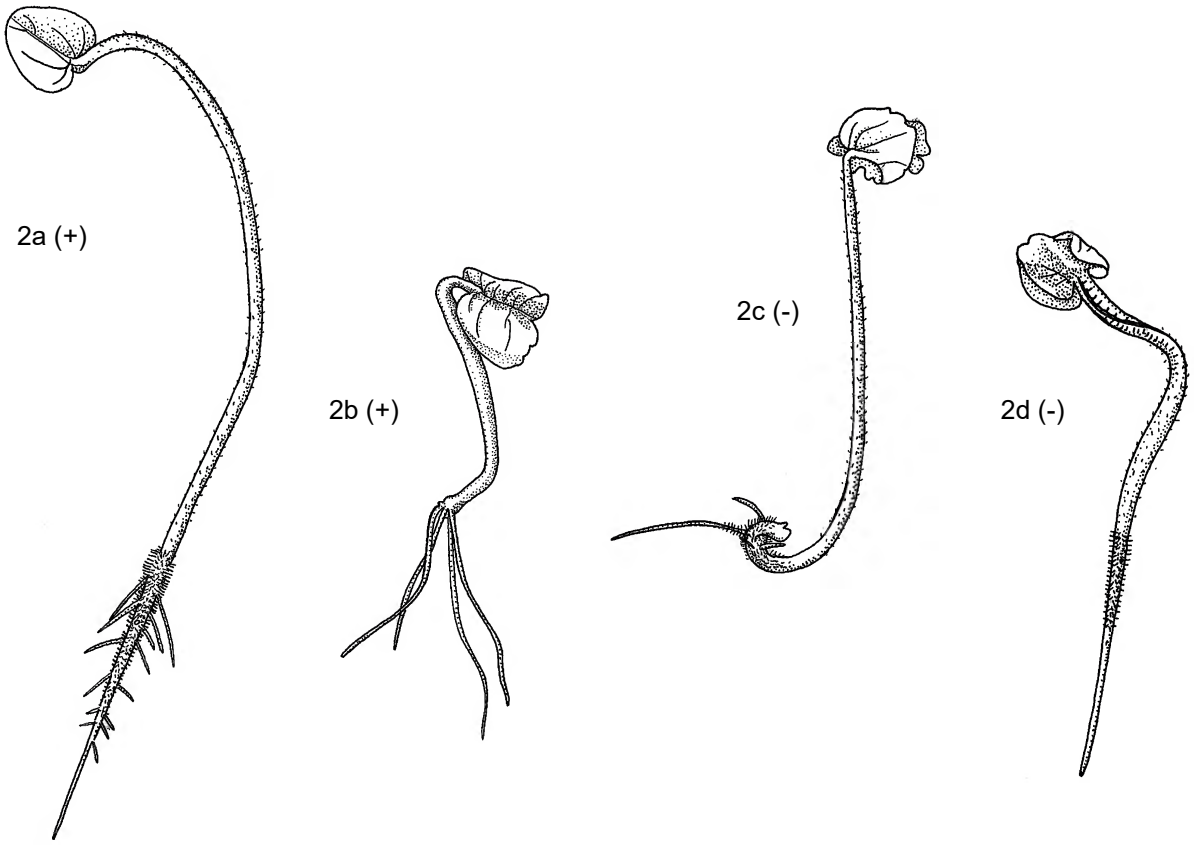


Fig. 2 Seedlings.

4.14.16 Poaceae, Grass Family I - Cereals

Avena spp., oat
Hordeum vulgare, barley
Secale cereale, rye
Triticum spp., wheat
x Triticosecale, triticale

General Description

Seedling type: Hypogeal monocot.

Food reserves: Endosperm. The scutellum is a modified cotyledon which is in direct contact with the endosperm. During germination the scutellum remains inside the seed absorbing nutrients from the endosperm and transferring them to the growing seedling.

Shoot system: The shoot consists of the coleoptile and enclosed leaves which grow from the meristematic region at their base and the mesocotyl. The shoot elongates and pushes through the media surface; the mesocotyl may elongate depending on the variety and light intensity, but is usually not discernible. Splitting of the coleoptile occurs naturally as a result of expansion of the leaves inside.

Root system: A primary root and seminal roots. The primary root is not readily distinguishable from the seminal roots, therefore all roots arising from the seed are referred to as seminal roots.

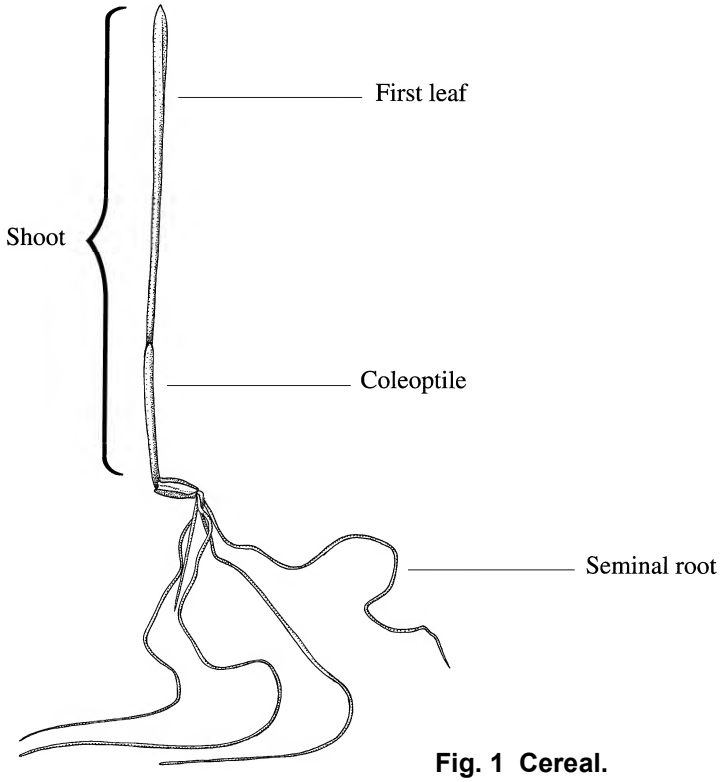


Fig. 1 Cereal.

Abnormal Seedling Description

Shoot:

- missing.
- no leaf.
- leaf extending less than halfway up into the coleoptile.
- Coleoptile split for more than one-third of the length from the tip
- Coleoptile damaged with leaf emerging through side split
- Coleoptile split near base with leaf bursting out
- leaf badly shredded or longitudinally split.
- thin, spindly, pale or watery.
- badly frost-damaged (characterized by graininess, spiral twisting and shredding, and loss of vigour).
- deep open cracks in the mesocotyl.
- (see note 1).

Root:

- less than one strong seminal root.

Seedling:

- decayed at point of attachment to the scutellum.
- one or more essential structures impaired as a result of decay from primary infection.
- albino.
- endosperm obviously detached from the root-shoot axis (e.g. kernel lifted away by the growing shoot).
- seedlings with badly thickened and shortened roots and shoots due to injury from chemical treatment (see note 2).

Notes:

1. Seedlings grown in the dark or in low intensity light will exhibit increased elongation of the coleoptile and in some cases the mesocotyl. In towels, there may be considerable twisting of the shoot.

2. Seedlings with badly thickened and shortened roots and shoots due to injury from chemical treatment are to be classified as abnormal. If such seedlings are difficult to evaluate on paper substrata, the interpretation must be based on the seedling performance in sand or soil-less organic growing media.

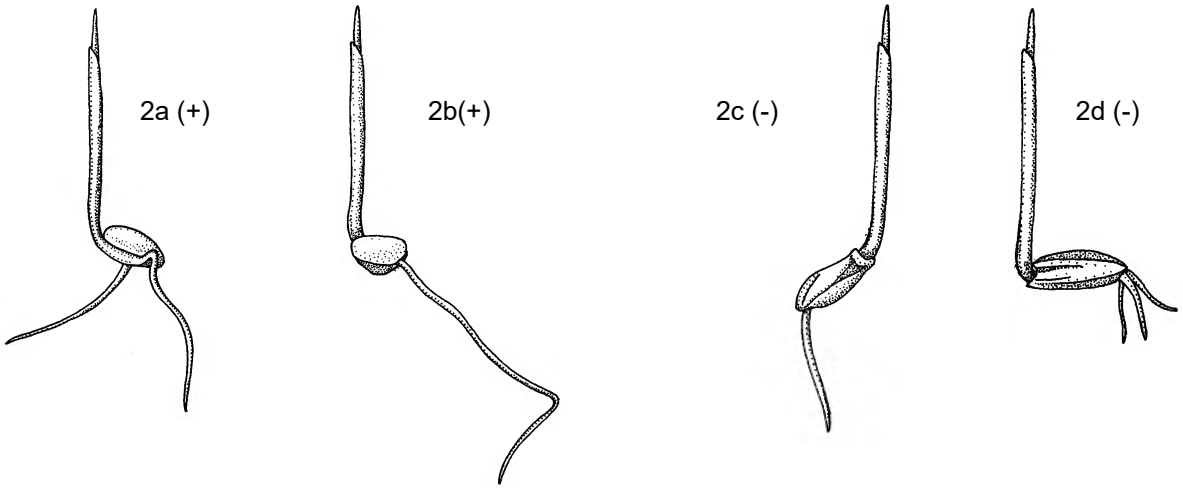


Fig. 2 Root defects.

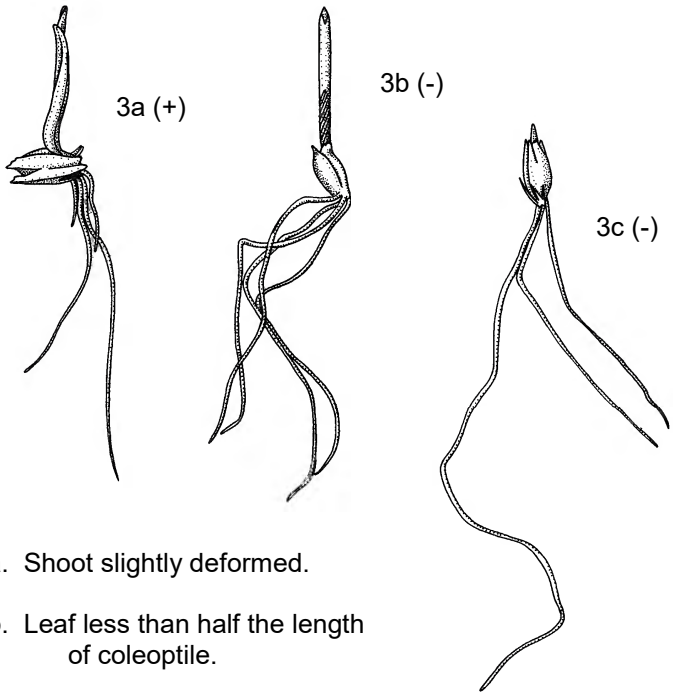
2a. Two strong seminal roots.

2b. One strong seminal root.

2c. Less than one strong seminal root.

2d. Less than one strong seminal root.

Fig. 3 Shoot defects.



3a. Shoot slightly deformed.

3b. Leaf less than half the length of coleoptile.

3c. Shoot not developing.

Fig. 4 Detached endosperm.

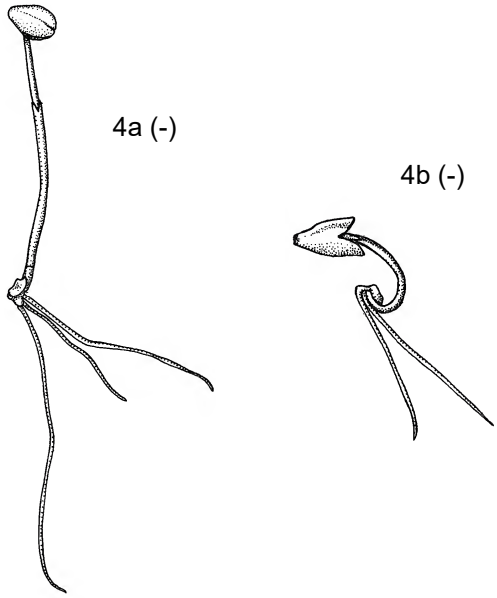
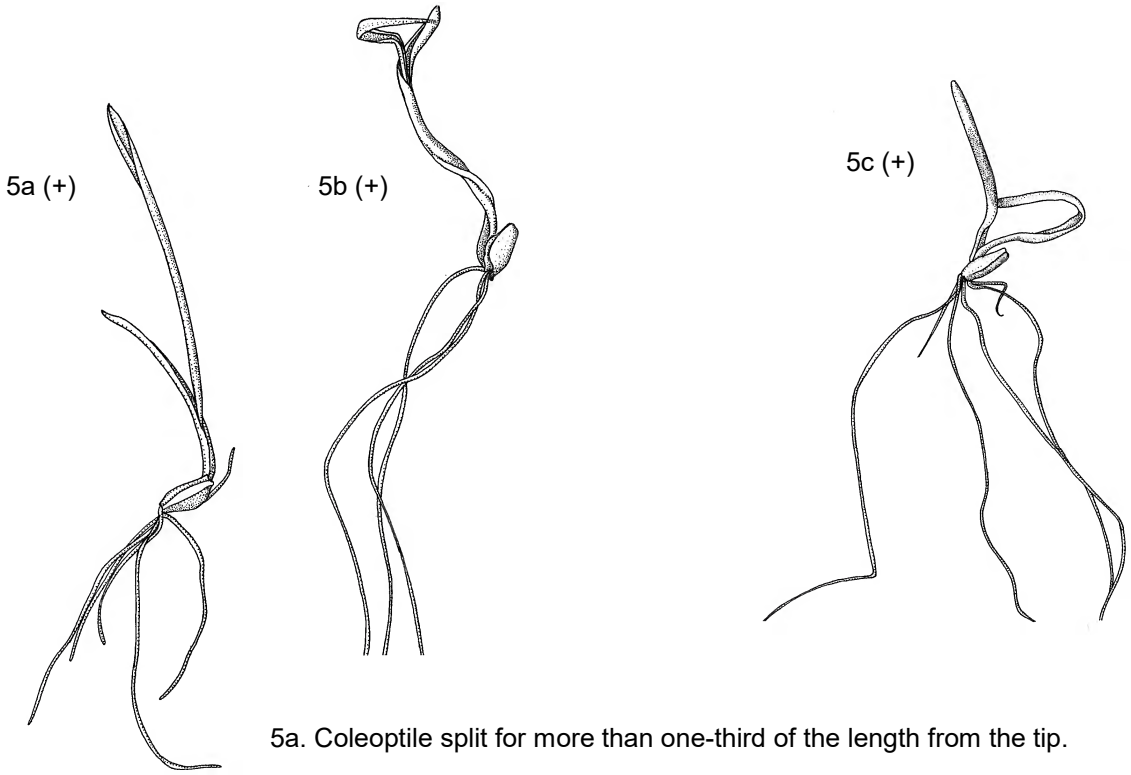


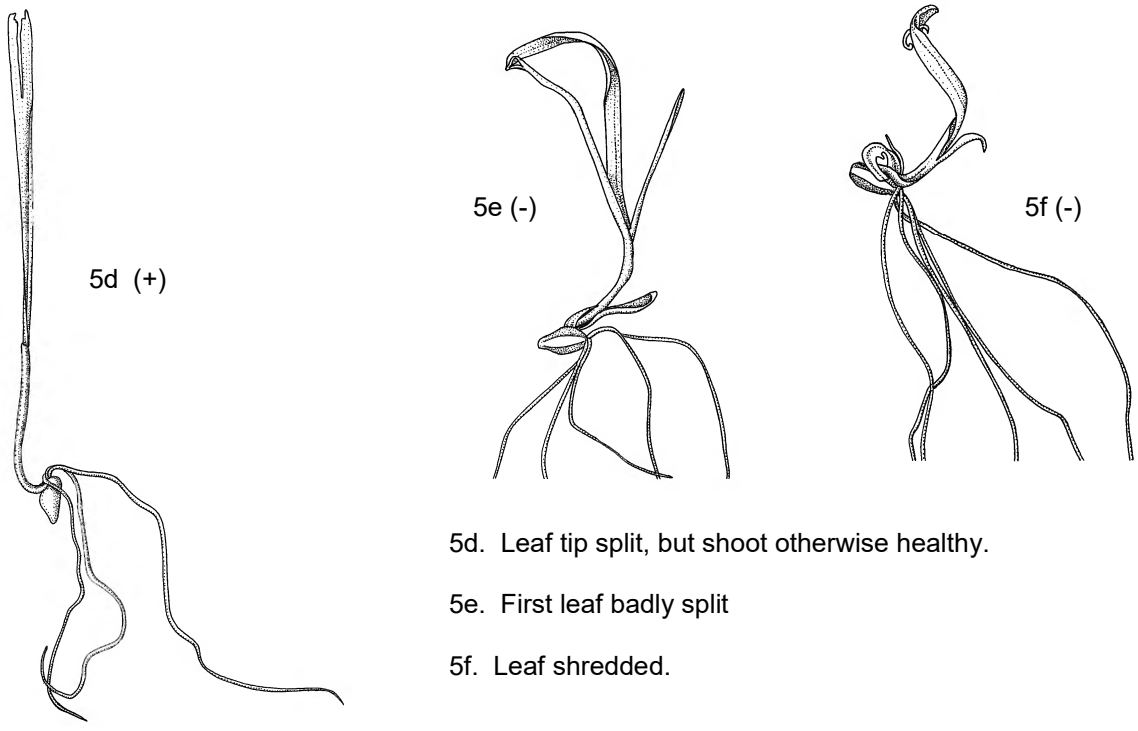
Fig. 5 Leaf defects.



5a. Coleoptile split for more than one-third of the length from the tip.

5b. Coleoptile damaged with leaf emerging through side split.

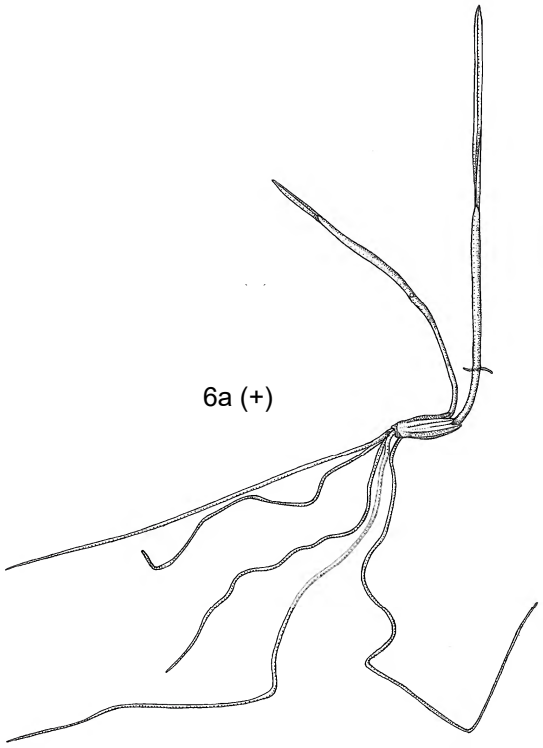
5c. Coleoptile split near base, with leaf bursting out.



5d. Leaf tip split, but shoot otherwise healthy.

5e. First leaf badly split

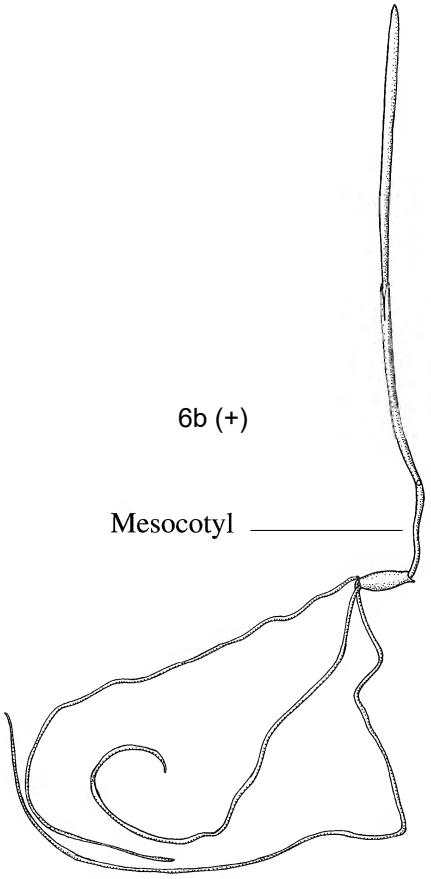
5f. Leaf shredded.



6a. Double oat.

6b. Seedling with elongated mesocotyl. Note that this gives the seedling the appearance of being “spindly”.

6c. Spiraling shoot, from being trapped in confined space such as towel or closely planted seeds.



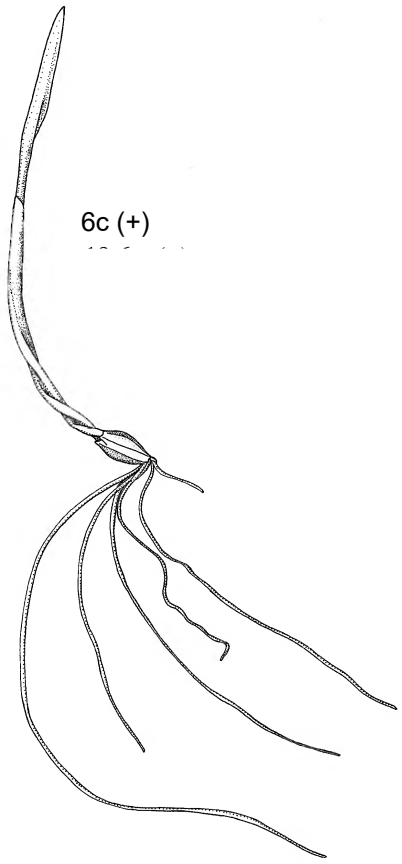


Fig. 6 Seedlings.

4.14.17 Poaceae, Grass Family III - Corn

Zea mays, corn

General Description

Seedling type: Hypogeal monocot.

Food reserves: Endosperm. The scutellum is a modified cotyledon which is in direct contact with the endosperm. During germination the scutellum remains inside the seed absorbing nutrients from the endosperm and transferring them to the growing seedling.

Shoot system: The shoot consists of the coleoptile and enclosed leaves which grow from the meristematic region at their base and the mesocotyl. The shoot elongates and pushes through the media surface. The mesocotyl usually elongates. Splitting of the coleoptile occurs naturally as a result of expansion of the leaves inside.

Root system: Strong primary root and seminal roots. Adventitious roots may start to develop from the mesocotyl or coleoptilar node within the test period.

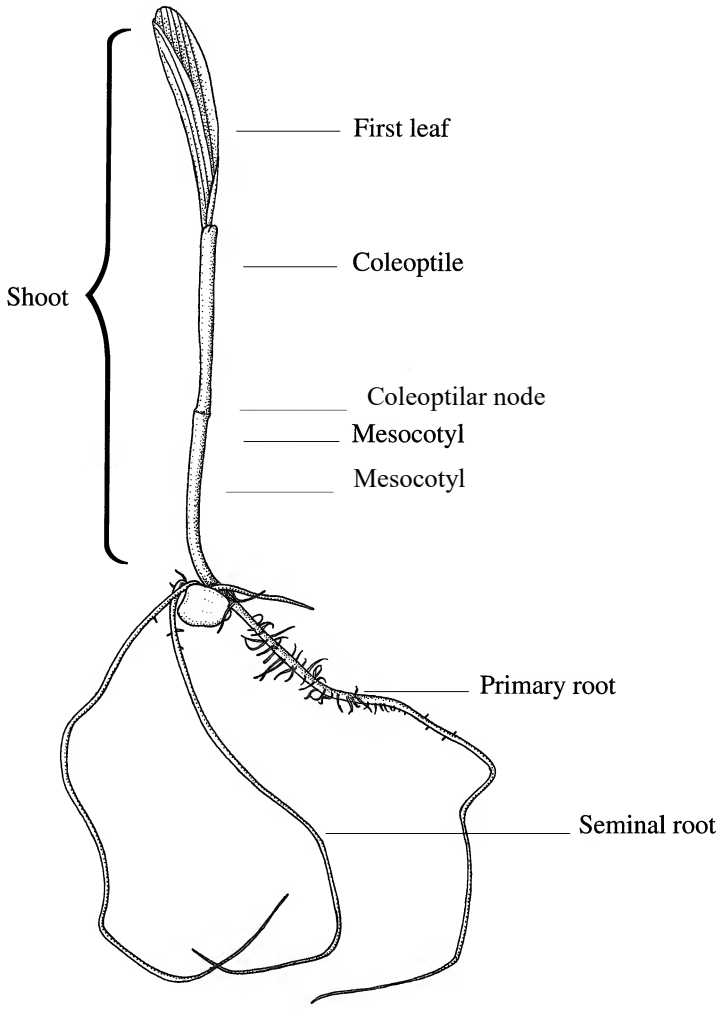


Fig. 1 Corn.

Abnormal Seedling Description

Shoot:

- missing.
- no leaf.
- leaf extending less than halfway up into the coleoptile (see note 3).
- leaf badly shredded or longitudinally split (see note 1).
- if the first leaf has emerged at time of evaluation, seedling is abnormal if the coleoptile has any of the following defects together with damage to the first leaf (see note 2).
  - coleoptile split for more than one-third of the length from the tip.
  - coleoptile strongly bent over
  - coleoptile tip damaged or missing
  - coleoptile split at any location below the tip
- if first leaf has not emerged at time of evaluation (see note 2).
  - tip of coleoptile damaged or missing
  - coleoptile split for more than one-third of the length from the tip
- leaf protruding below the tip of the coleoptile.
- thin, spindly, pale or watery.
- deep open cracks in the mesocotyl.

Root:

- none.
- weak, stubby or missing primary root with weak seminal roots.

Seedling:

- decayed at point of attachment to the scutellum.
- one or more essential structures impaired as a result of decay from primary infection.
- albino.

Notes:

1. Seedlings grown in the dark or in low intensity light will exhibit increased elongation of the coleoptile and mesocotyl. In towels, there may be considerable twisting of the shoot system. Overcrowding may cause splitting of the coleoptile and leaves.

2. Occasionally in the sand test and often in the rolled towel test, the leaf will not have emerged through the tip of the coleoptile by the end of the full germination period (7 days). If the leaf has not emerged at the time of final evaluation, seedlings with a split in the coleoptile for more than one-third of the length from the tip and seedlings with the coleoptile tip missing or damaged must be classed abnormal. An exception to this rule may be made when it is clearly evident that the splitting is due to pressure caused by restricted growth within the substrate. When the first leaf has emerged through the tip of the coleoptile at the time of evaluation, it is possible to use damage to the leaf as evidence that damage to the coleoptile is significant. Seedlings with the coleoptile defects may be classed as normal when the first leaf is intact or only slightly damaged.

3. When determining whether or not the leaf has attained half the length of the coleoptile or more, the measurement is to be taken from the base of the coleoptile (coleoptilar node) and not necessarily from the base of the shoot (which may include an elongated mesocotyl).

4. A twisted and curled shoot bound by a tough seed coat may be considered normal.

5. Seedlings from frost-damaged seeds may be characterized by grainy coleoptiles and spirally twisted leaves as well as decay at the point of attachment to the scutellum.

6. Flat seeds germinate faster than round seeds. It may be necessary to extend the test by two days for round seeds.

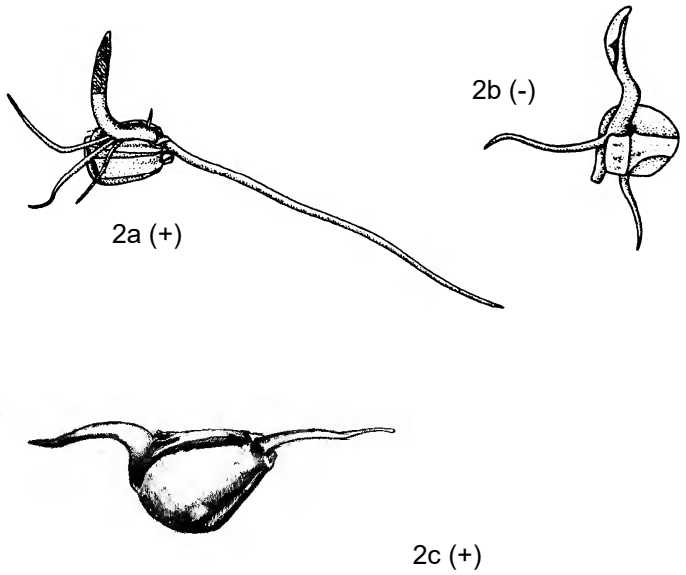


Fig. 2 Small seedlings.

2a. Late-germinating seedling

2b. Shoot damaged and weak roots.

2c. Shoot and root the length of the kernel.

Fig. 3 Split leaves.

3a. Leaf damaged, coleoptile tip damaged (see also Fig. 7).
3b. Leaf badly split.
3c. Leaf shredded.
3d. Leaf damaged.

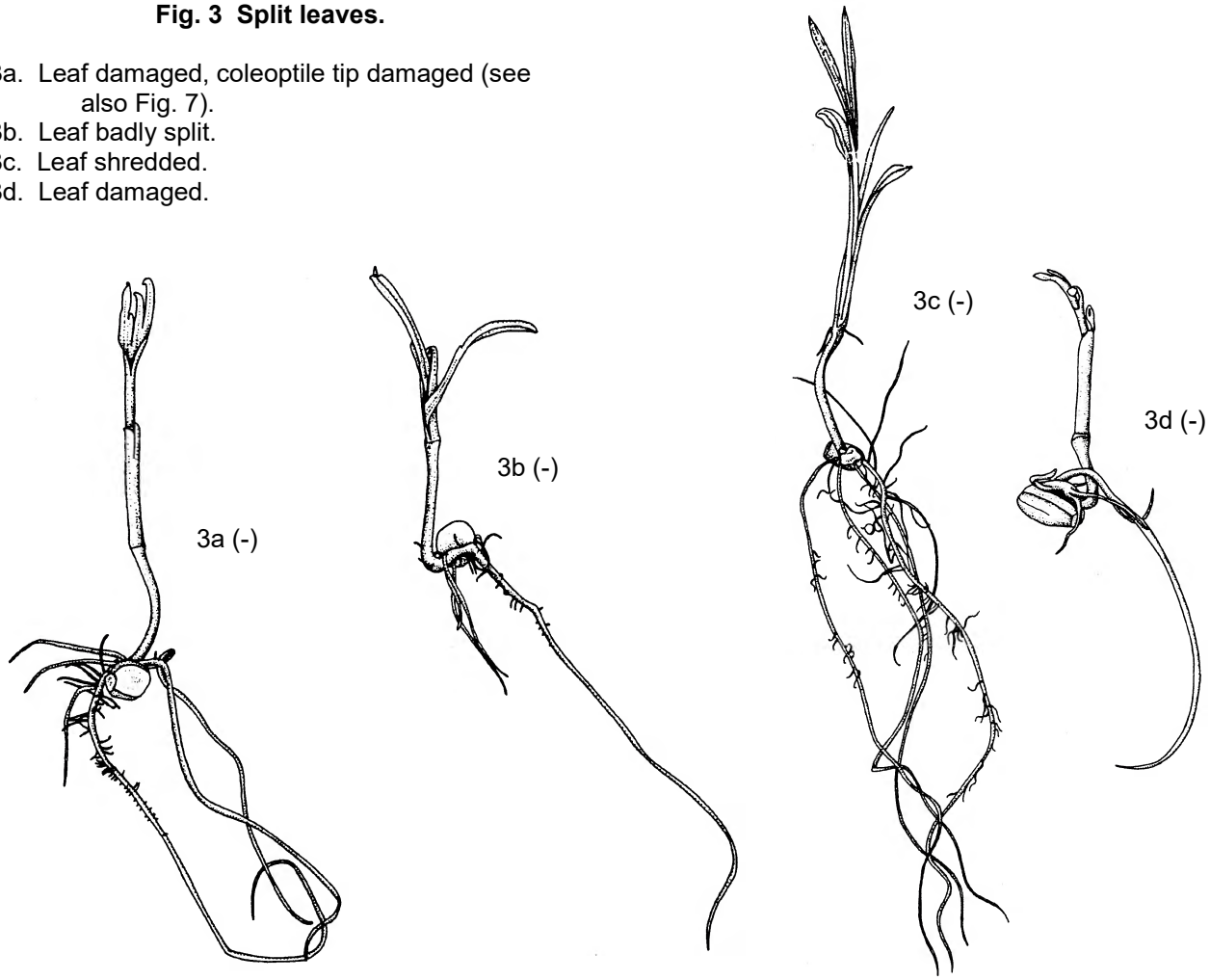


Fig. 4 Shoot defects.

4a. Coleoptile split, but seedling otherwise healthy.

4b. coleoptile split, leaf curled due to test conditions.

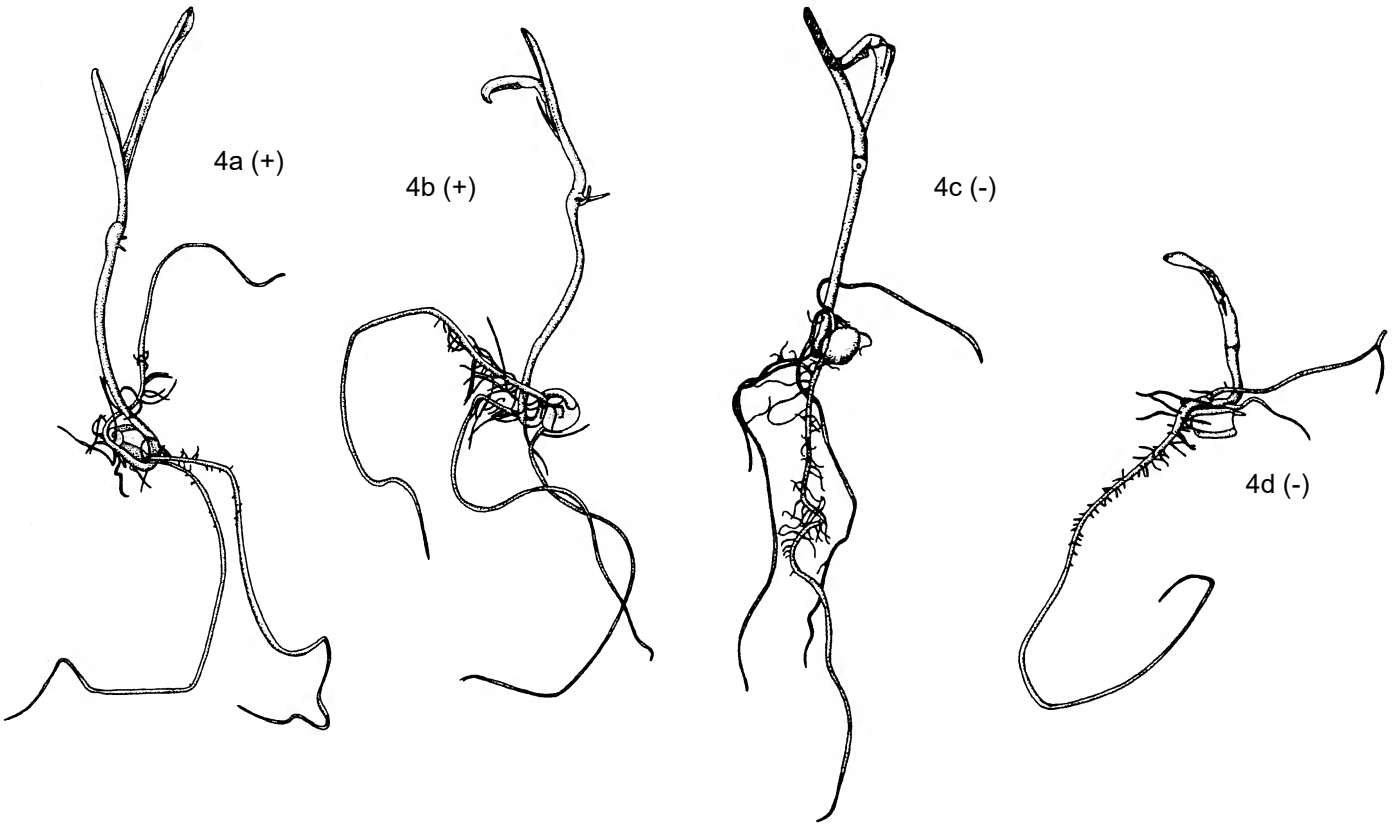
4c. Leaf emerged from base of coleoptile.

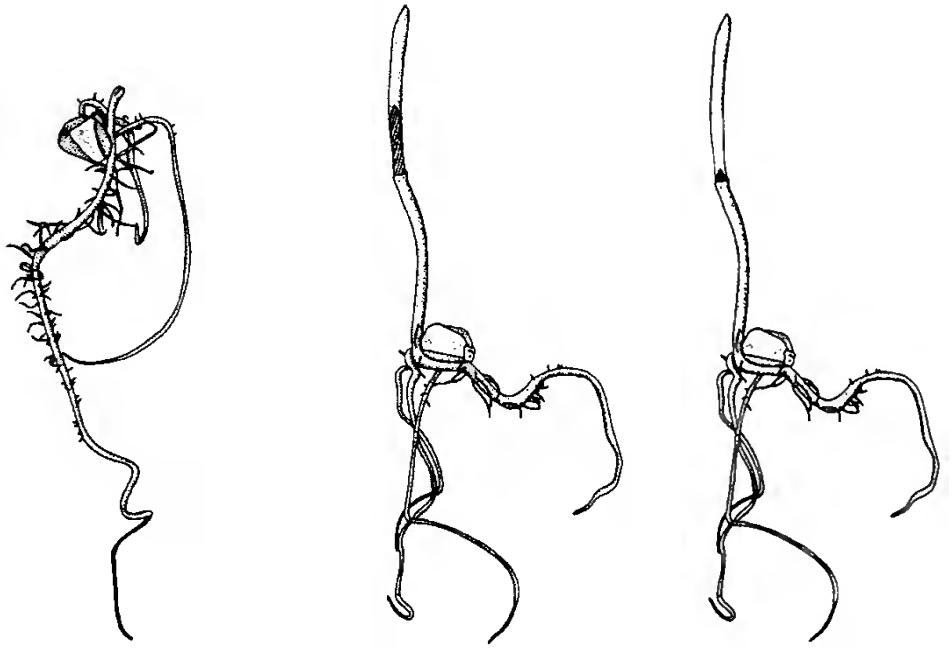
4d. Damaged coleoptile with shredded leaf.

4e. Shoot damaged and not developing.

4f. Leaf less than half the length of coleoptile

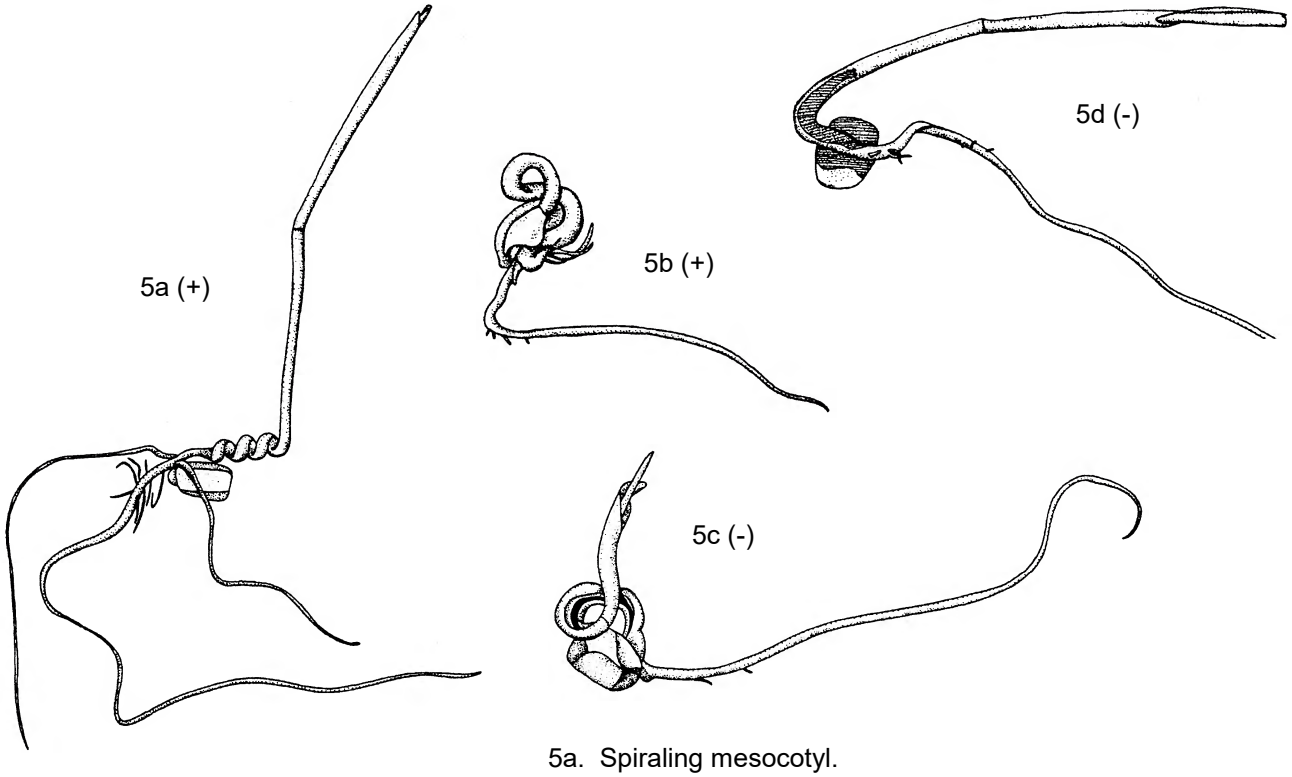
4g. Leaf meristematic tissue present or absent, but no evident growth of leaves in the coleoptile





4e (-) 4f (-) 4g(-)

Fig. 5 Mesocotyl defects.



5a. Spiraling mesocotyl.

5b. Shoot trapped in seed coat, but otherwise healthy.

5c. Deep mesocotyl lesion.

5d. Decay at point of attachment to scutellum.

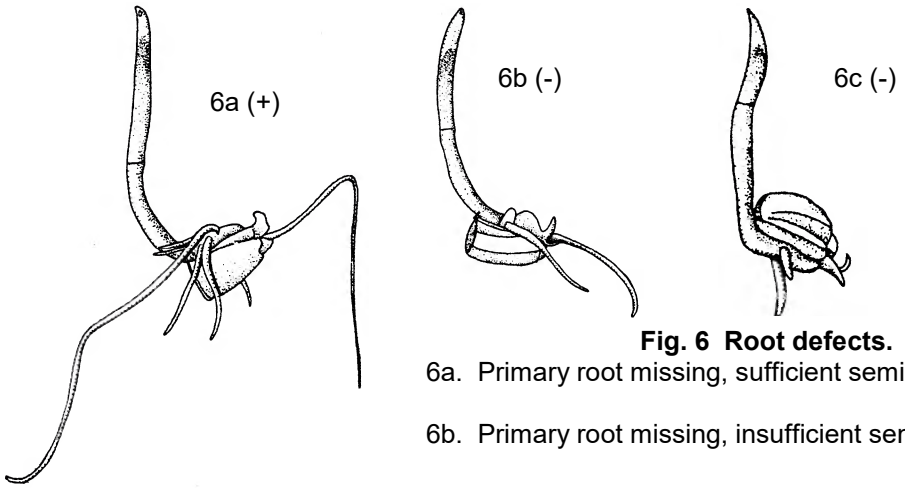


Fig. 6 Root defects.

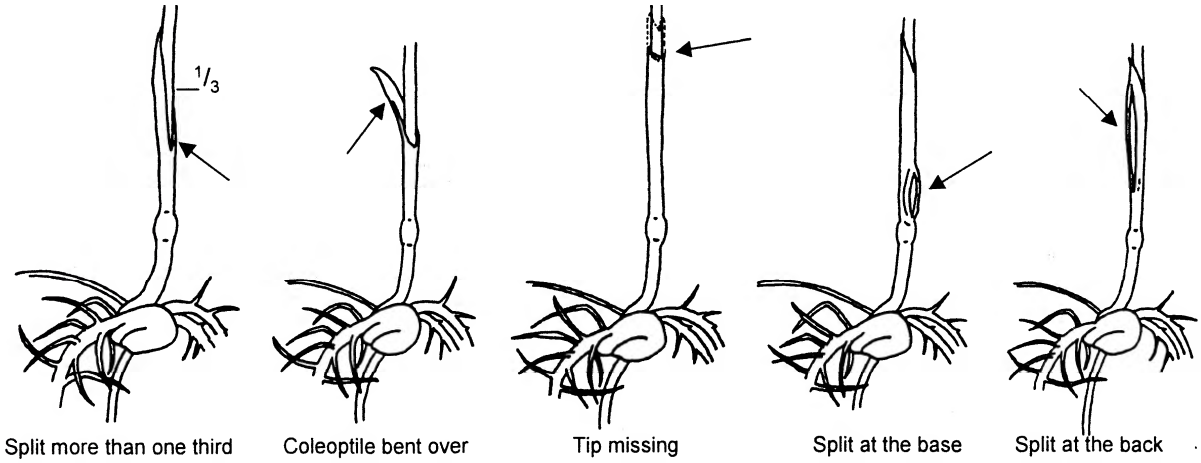
6a. Primary root missing, sufficient seminal roots.

6b. Primary root missing, insufficient seminal roots.

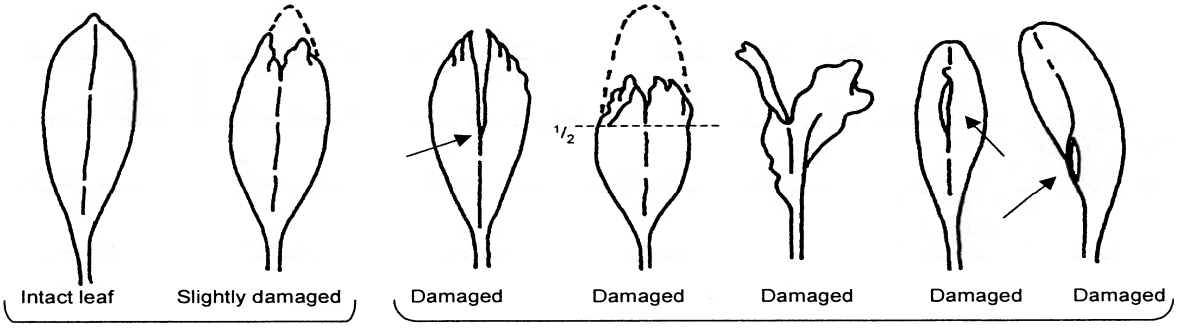
6c. Insufficient roots.

Fig. 7 Coleoptile defects.

7a. Seedlings are normal if the first leaf is intact or only slightly damaged, as defined in Figure 7b. Seedlings are abnormal if first leaf is damaged, as defined in Figure 7b.



7b. Definition of intact, slightly damaged and damaged first leaf, for evaluation of seedlings with coleoptile defects.



4.14.18 Poaceae, Grass Family IV - Sorghum

Sorghum spp., sorghum, sudan grass and sorghum-sudan grass hybrids

General Description

Seedling type: Hypogeal monocot.

Food reserves: Endosperm. The scutellum is a modified cotyledon which is in direct contact with the endosperm. During germination the scutellum remains inside the seed absorbing nutrients from the endosperm and transferring them to the growing seedling.

Shoot system: The shoot consists of the coleoptile and enclosed leaves which grow from the meristematic region at their base and the mesocotyl. The shoot elongates and pushes through the media surface; the mesocotyl usually elongates. Areas of natural, reddish pigmentation may develop on the mesocotyl and coleoptile. Splitting of the coleoptile occurs naturally as a result of expansion of the leaves inside.

Root system: A long primary root, usually with secondary roots developing within the test period. Adventitious roots arising from the mesocotyl and coleoptilar node may start development within the test period. Areas of natural, reddish pigmentation may develop on the root.

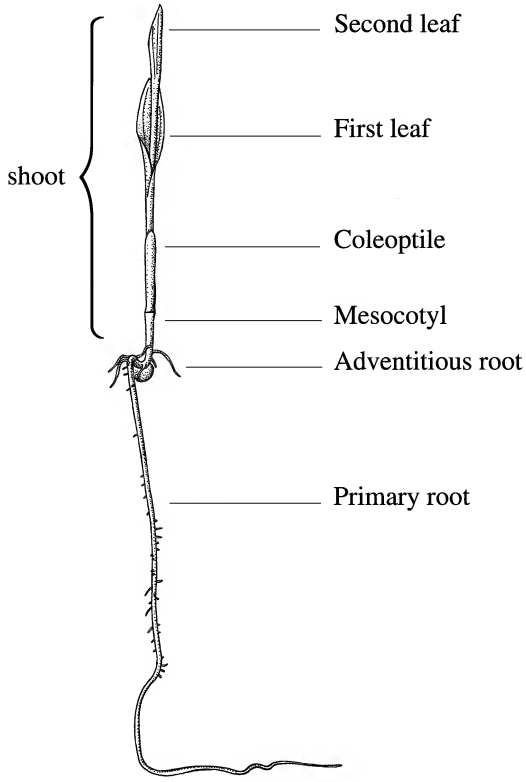


Fig. 1 Sorghum

Abnormal Seedling Description

Shoot:

- missing.
- no leaf.
- leaf extending less than halfway up into the coleoptile.
- leaf badly shredded or longitudinally split.
- thin, spindly, pale or watery (in comparison with other seedlings in the test).
- deep open cracks in the mesocotyl.
- (see notes 1, 2 and 3).

Root:

- none.
- damaged or weak primary root with less than two strong secondary roots.

Seedling:

- decayed at point of attachment to the scutellum.
- one or more essential structures impaired as a result of decay from primary infection.
- albino.

Notes:

1. Splitting of the coleoptile occurs naturally as a result of expansion of the leaves inside. The condition of the coleoptile is not to be considered as an evaluation factor on its own; however, damage to the coleoptile is a signal that the other shoot structures must be examined closely to determine if they have been damaged.

2. Seedlings with red coloration on or in the roots or coleoptiles caused by natural pigmentation are to be considered normal.

3. Seedlings from frost-damaged seeds may be characterized by grainy coleoptiles and spirally twisted leaves as well as decay at the point of attachment to the scutellum.

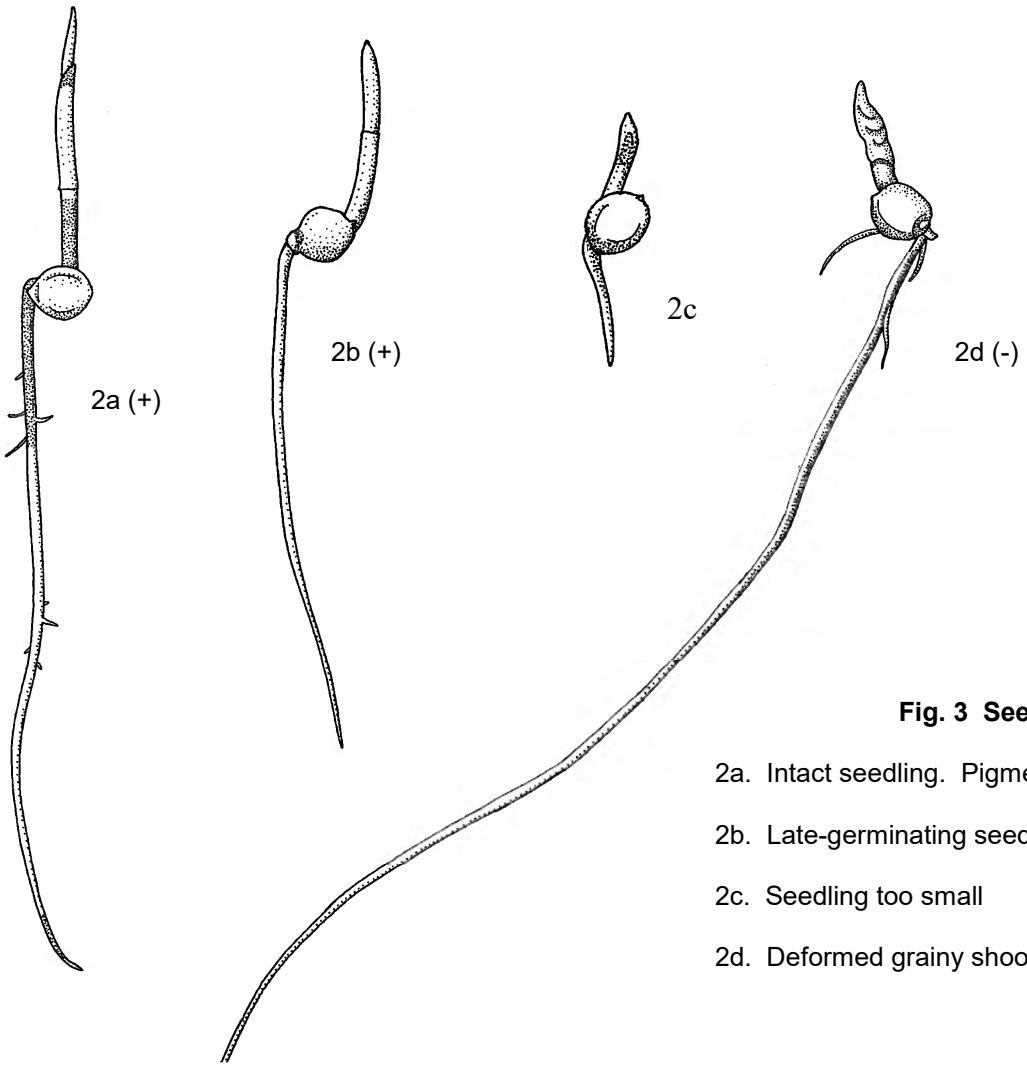


Fig. 3 Seedlings.

2a. Intact seedling. Pigmentation normal.

2b. Late-germinating seedling

2c. Seedling too small

2d. Deformed grainy shoot.

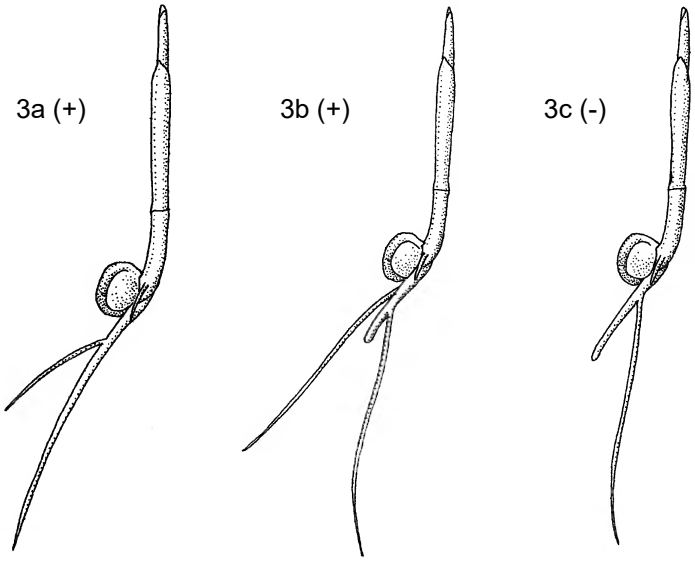


Fig. 3 Root defects.

3a. Primary root long enough.

3b. Stubby primary root, with sufficient secondary roots.

3c. Stubby primary root, with insufficient secondary roots.

4.14.19 Poaceae, Grass Family V - Other Kinds

All grass species listed in Section 4.6.2 Table 5, other than barley, corn, oats, rye, sorghum, triticale and wheat.

General Description

Seedling type: Hypogeal monocot.

Food reserves: Endosperm. The scutellum is a modified cotyledon which is in direct contact with the endosperm. During germination the scutellum remains inside the seed absorbing nutrients from the endosperm and transferring them to the growing seedling.

Shoot system: The shoot consists of the coleoptile and enclosed leaves which grow from the meristematic region at their base and the mesocotyl. The shoot elongates and pushes through the media surface. The mesocotyl usually does not elongate significantly in most species, but may in some (e.g. tall oatgrass). Splitting of the coleoptile occurs naturally as a result of expansion of the leaves inside.

Root system: At the beginning of germination, the radicle breaks through the coleorhiza and seed coat and develops into a long primary root. Secondary or adventitious roots usually do not develop within the test period.

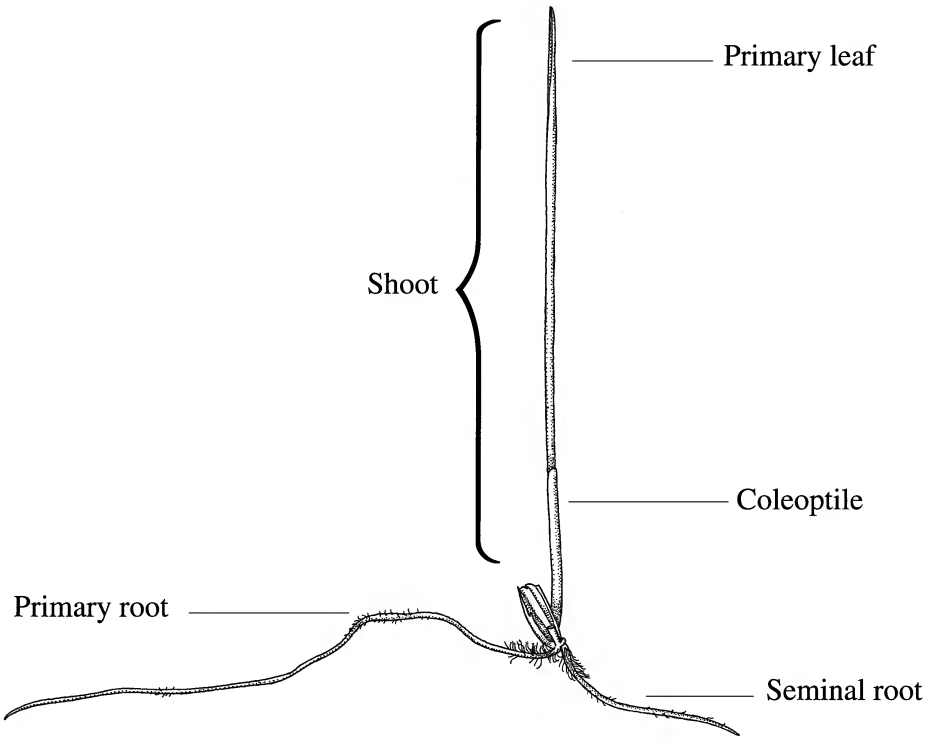


Fig. 1 Ryegrass.

Abnormal Seedling Description

Shoot:

- missing.
- short, thick and grainy.
- no leaf.
- leaf extending less than halfway up into the coleoptile.
- leaf badly shredded or longitudinally split.
- thin, spindly, pale or watery.
- deep open cracks in the mesocotyl.
- (see also notes 3 and 6)

Root:

- missing or defective primary root even if other roots are present (see note 2).
- spindly, stubby or watery primary root (see note 1; for Kentucky bluegrass, see note 5).

Seedling:

- decayed at point of attachment to the scutellum.
- one or more essential structures impaired as a result of decay from primary infection.
- albino.
- yellow (when grown in light).
- endosperm obviously detached from the root-shoot axis (e.g. kernel lifted away by the growing shoot).

Notes:

1. The use of a potassium nitrate solution is recommended for breaking dormancy in certain species (see Section 4.6.2 Table 5). Its use may cause shortened roots and promote fungal growth. Retests on top of soil-less organic growing media in closed petri dishes are recommended to aid in interpretation of short roots which appear to be caused by the use of potassium nitrate.

2. In certain species the primary root may not be readily visible because it is coiled inside the tightly fitting lemma and palea. At the time of evaluation, the glumes must be removed and the root observed. Such seedlings are to be classified as normal if the root has developed.

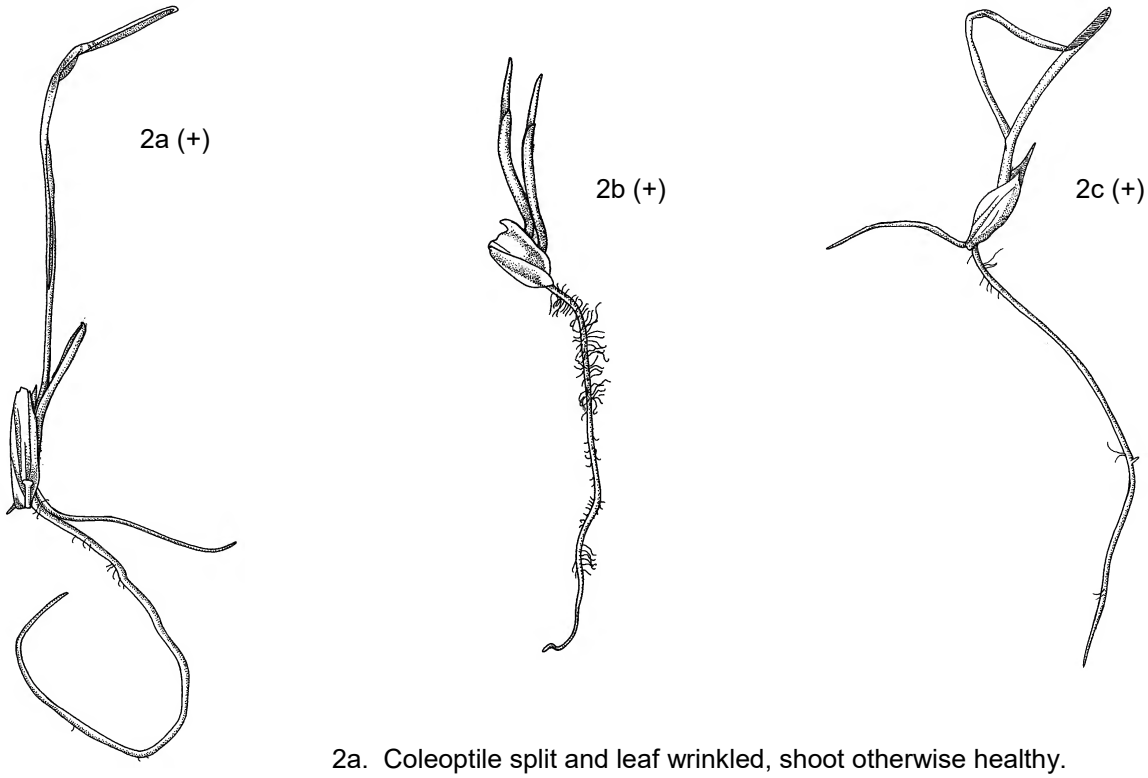
3. Splitting of the coleoptile occurs naturally as a result of expansion of the leaves inside. The condition of the coleoptile is not to be considered as an evaluation factor on its own; however, damage to the coleoptile is an indication that the other shoot structures must be examined closely to determine if they have been damaged.

4. The temperature alternations and prechilling treatments listed in the rules are specific. It is stressed that lack of correct temperature control may cause grass seeds to exhibit erratic germination, to show no growth at all or to cause secondary dormancy.

5. For Kentucky bluegrass, seedlings with a primary root 2 mm or more in length are to be classified as normal.

6. Seedlings from frost-damaged seeds may be characterized by grainy coleoptiles and spirally twisted leaves as well as decay at the point of attachment to the scutellum.

Fig. 2 Shoot defects.



2a. Coleoptile split and leaf wrinkled, shoot otherwise healthy.

2b. Double shoot.

2c. Coleoptile split below the tip and leaf busting out.

2d. Badly split or shredded leaf.

2e. Leaf less than half the length of the coleoptile.

2f. Short grainy shoot.

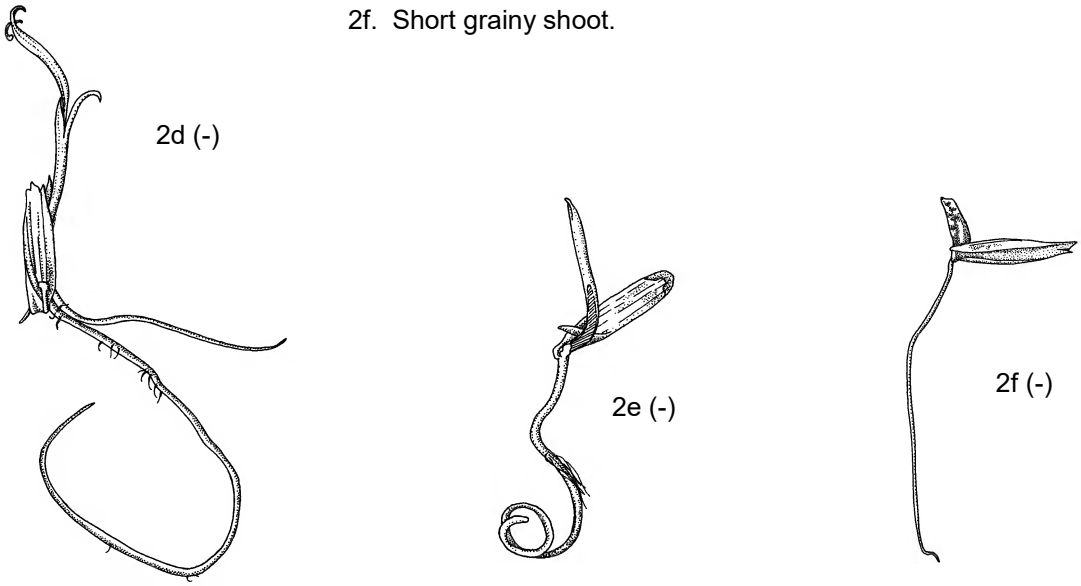
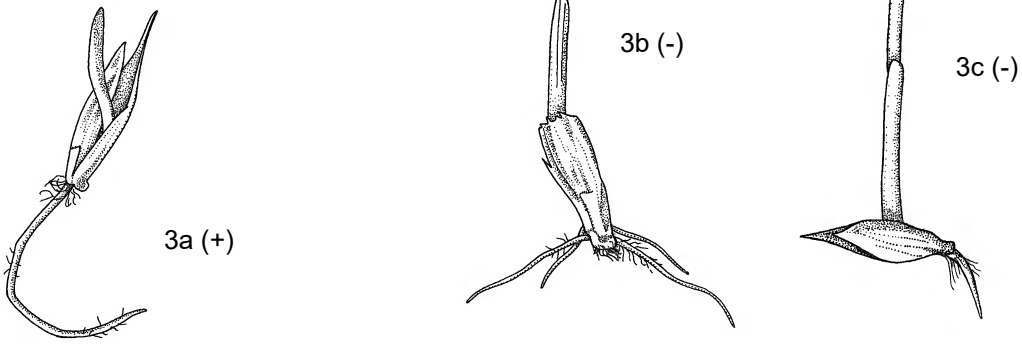


Fig. 3 Root defects.

3a. Late-germinating seedling

3b. Missing primary root (note shriveled leaf tip).

3c. Stubby primary root.



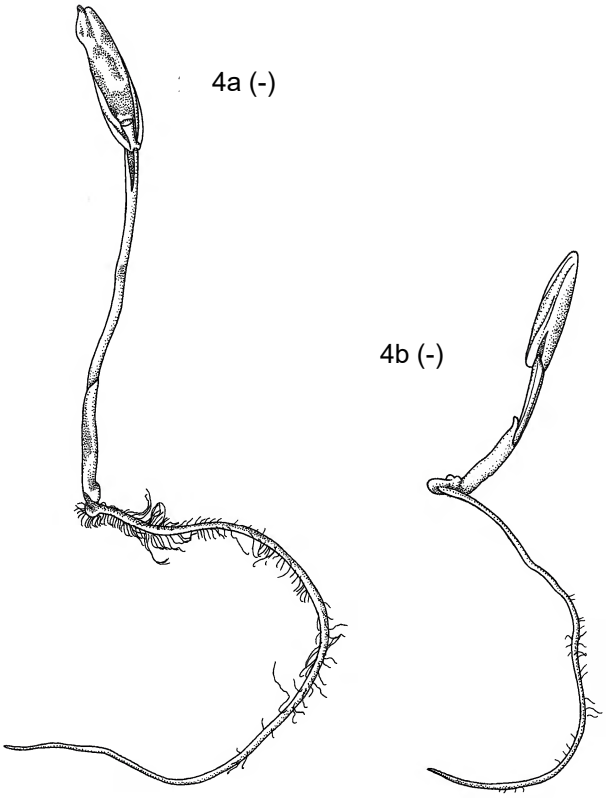


Fig. 4 Detached endosperm.

4a. Endosperm detached from the root-shoot axis and seedling strong.

4b. Endosperm detached from the root-shoot axis and seedling weak.

4.14.20 Polygonaceae, Knotweed Family

Fagopyrum spp., buckwheat
Rheum xhybridum, rhubarb
Rumex acetosa, sorrel

General Description

Seedling type: Epigeal dicot.

Food reserves: Cotyledons, starchy endosperm

Shoot system: The hypocotyl elongates carrying the cotyledons above the media surface. The epicotyl usually does not show any development within the test period.

Root system: A primary root with secondary roots developing within the test period for some species.

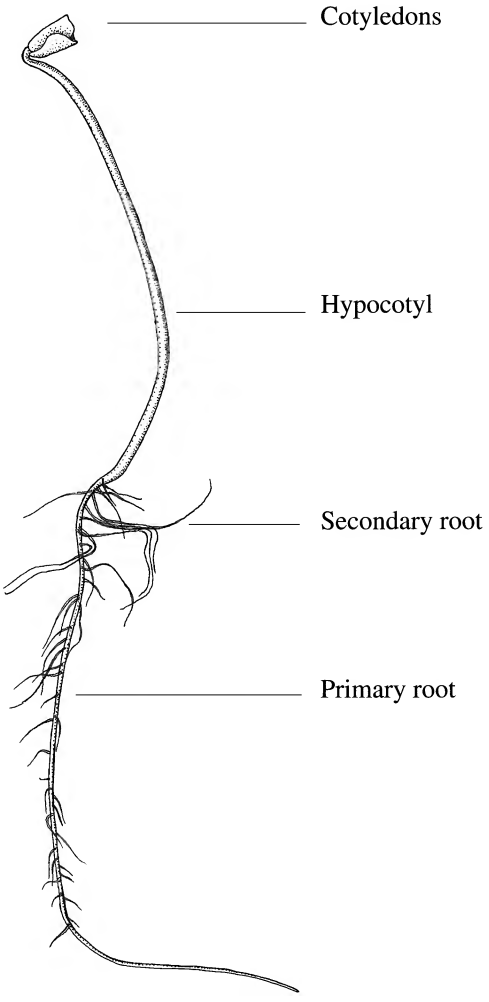


Fig. 1 Buckwheat.

Abnormal Seedling Description

Cotyledons:
- less than half of the original cotyledon tissue remaining attached.
- less than half of the original cotyledon tissue free of necrosis or decay.

Epicotyl:
- missing (may be assumed to be present if cotyledons are intact).

Hypocotyl:
- deep open cracks or grainy lesions extending into the conducting tissue.
- malformed, such as markedly shortened, curled or thickened.

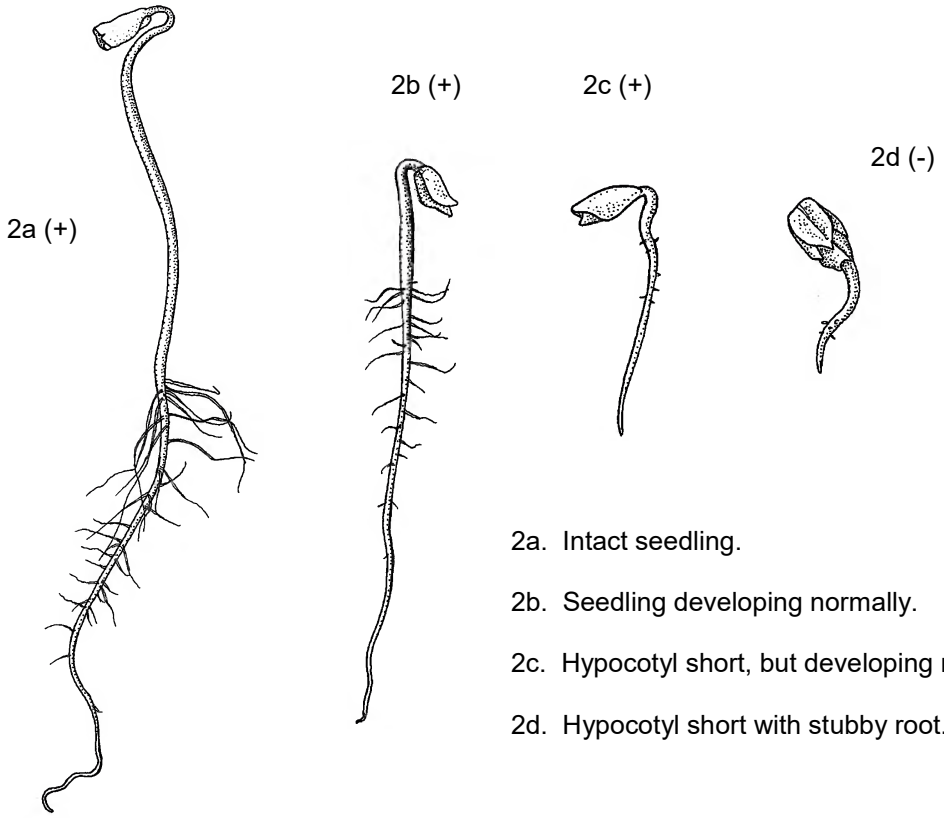
Root:
- none.
- weak, stubby or missing primary root with weak secondary or adventitious roots.

Seedling:
- one or more essential structures impaired as a result of decay from primary infection.
- albino.

Notes:

None

Fig. 2 Small seedlings.



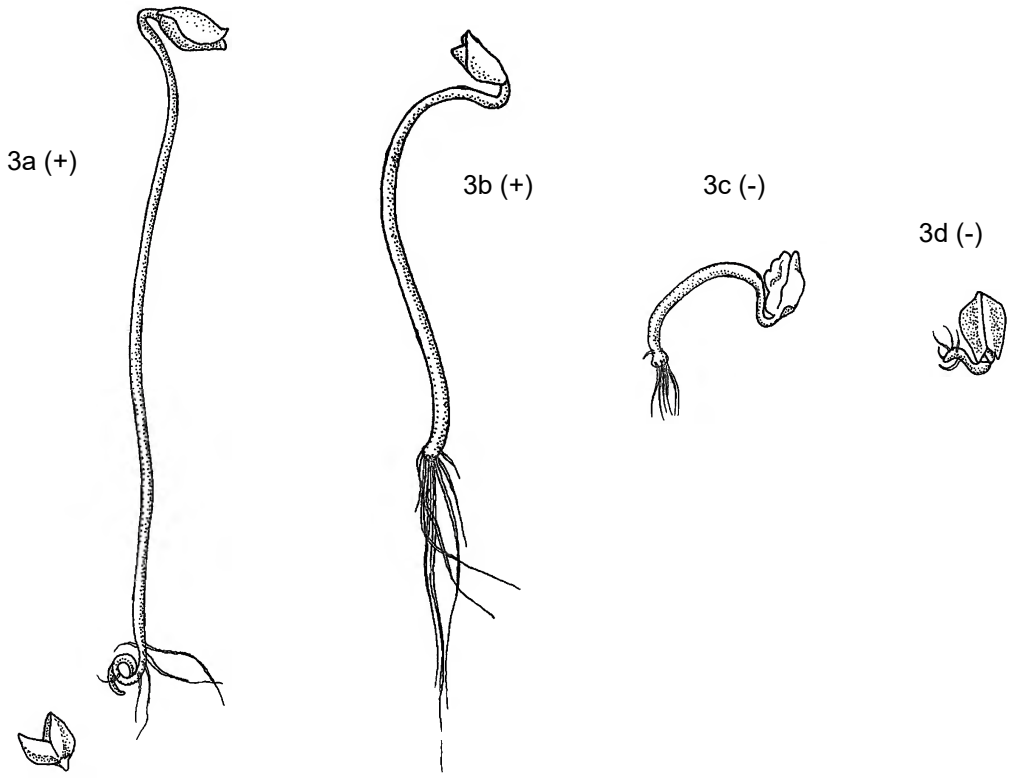
2a. Intact seedling.

2b. Seedling developing normally.

2c. Hypocotyl short, but developing normally.

2d. Hypocotyl short with stubby root.

Fig. 3 Root defects.



3a. Root coiled inside seed coat.

3b. Primary root damaged or missing, sufficient secondary or adventitious roots.

3c. Insufficient roots.

3d. Insufficient roots and short hypocotyl.

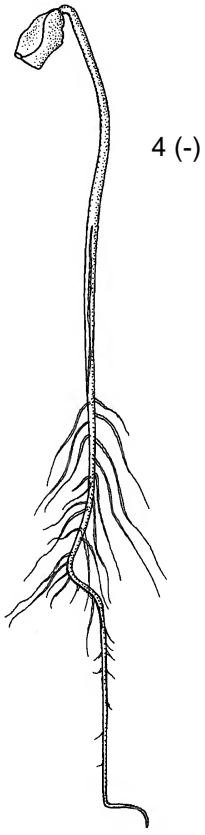


Fig. 4 Deep hypocotyl lesion

4.14.21 Miscellaneous Agricultural and Horticultural Families

Apiaceae, carrot family - carrot, celery, celeriac, chervil, dill, parsley, parsnip
Boraginaceae, borage family - borage
Lamiaceae, mint family - sage, savory, thyme
Solanaceae, nightshade family - eggplant, tomato, pepper, tobacco
Valerianaceae, valerian family - cornsalad

General Description

Seedlings are considered normal if they possess those essential structures that are indicative of their ability to produce a plant under favourable conditions.

Abnormal Seedling Description

Cotyledons:
- less than half of the original cotyledon tissue remaining attached.
- less than half of the original cotyledon tissue free of necrosis or decay.

Epicotyl:
- missing (may be assumed to be present if the cotyledons are intact).

Hypocotyl:
- malformed, such as markedly shortened, curled or thickened.
- deep open cracks extending into the conducting tissue.
- watery.

Root:
- none.
- **In kinds other than carrot, celeriac and parsnip:** missing or stubby primary root with weak secondary or adventitious roots.
- **In carrot, celeriac and parsnip:** missing or stubby primary root (even if secondary roots are present).

Notes:

None

5.0 EMBRYO TEST FOR TRUE LOOSE SMUT OF BARLEY

5.1 INTRODUCTION

This chapter provides the definitions, descriptions, working sample sizes and procedures used for providing quantitative data on the incidence of true loose smut infected barley embryos as required for grading barley under GradeTable II.

The determination of the incidence of the disease is based on the number of embryos of barley seed that contain the mycelium of *Ustilago nuda,* the causal agent of true loose smut of barley.

5.2 DEFINITIONS

Hypha: (pl. *hyphae*) A single thread of a fungus mycelium

Intact embryo: Is an embryo that has the plumular area and the scutellum area intact.

Mycelium: (pl. *mycelia*) A mass of hyphae forming the body of a fungus.

Septate: Having cross walls.

MSDS: Material Safety Data Sheets

5.3 CAUTIONS

a. The chemicals used in this test are corrosive and/or toxic. Analysts should read and understand the MSDS for these chemicals (sodium hydroxide, trypan blue, ethanol, lactic acid, glycerin). The safety directions in the MSDS should be followed at all times. Analysts should be familiar with the principles of good Laboratory Practice. Analysts should wear the appropriate personal protective equipment when handling chemicals.

b. There may be times that the seed is chemically treated (Treated Seed). Analysts should read and understand the MSDS for these seed treatments. Every effort should be made to conduct the test on the untreated seed. If this is not possible the treated seed as received must be tested.

5.4 SOURCE AND NUMBER SEEDS FOR THE EMBRYO TEST

a. Source of Seeds. Seeds must be taken directly from the submitted sample after careful mixing as described in Section 2.2. The seeds must be taken without discrimination. Sufficient seed must be selected to ensure that there are 400 intact embryos for evaluation.

b. Number of Seeds. Given the current standards set out in Grade Table II at least 400 embryos must be examined for true loose smut. Therefore, in order to ensure that 400 intact embryos will be available for examination, 600-800 seeds are required for the extraction procedure. The seeds can be counted out manually, with the use of a vacuum counter or by a volume measure known to provide a sufficient number of seeds for the test.

5.5 PREPARATION OF REAGENTS

It is not necessary to use distilled water for the preparation of NaOH solution. Distilled water must be used for preparing saturated solutions of Trypan Blue

5.5.1 Sodium Hydroxide Stock Solutions

a. 50% Stock Solution (Reagent A).

Add 500 grams NaOH pellets to 500 mL H_2O.
Caution: If using NaOH pellets in preparing stock solutions extreme caution must be exercised when adding pellets to water as a great deal of heat is produced.

A safer and quicker approach is to begin with a commercially prepared stock solution of 50% NaOH. Heat is produced when water is added to this solution but to a much lesser extent.

b. 5% NaOH Working Solution (Reagent B).

Add 100 mL of Reagent A to 900 mL of H_2O.

Alternatively, carefully add 50 grams of NaOH pellets to 950 mL H_2O.

NOTE: The five percent sodium hydroxide (NaOH) solutions may be prepared in advance and stored or alternatively, they may be prepared and used immediately.

5.5.2 Trypan Blue

Trypan blue is an intense blue dye readily absorbed by fungal mycelium.

a. Stock solution: 10% trypan blue (saturated solution) add 2g of trypan blue to 20 mL distilled water; or 10g of trypan blue to 100 mL distilled water.

The saturated solution is prepared by slowly adding the suggested amount of trypan blue powder to the distilled water and mixing thoroughly until no more dye will go into the solution. Decant the solution and store away from light. (Storage in a dark bottle wrapped in tinfoil will preserve this reagent for long periods). If traces of trypan blue can be observed crystallizing on the sides of the bottle, the solution must be discarded at once, as the crystals will interfere with the test.

b. Working Solution – the following options can be followed:

2 mL of trypan blue stock solution is added to 500 mL bottle of 85% lactic acid, mix well

0.2g of trypan blue (powder) is added to 500 mL of 85% lactic acid (directly into the bottle) and then shaken to mix.

0.45 mL (2-3 drops) of stock solution added to 25 mL of 85% lactic acid

It is recommended that the option chosen for the preparation of the reagents for the test be based on the numbers of tests conducted. The best practice is to mix quantities of working solutions that will be used within short time frames.

5.6 EMBRYO EXTRACTION

Place the seed (600-800 seeds) in a 1L beaker containing a minimum of 600 mL of the NaOH working solution (Reagent B). To prevent accidental contact with the strong NaOH solution a cover should be placed over the beaker. Incubate overnight at room temperature.

After incubation add a small quantity of tepid water to the beaker and agitate gently to re-suspend embryos and chaff. Gently pour the contents onto the top screen (6 mesh) of a series of Tyler Standard Test Sieves (6, 8, 14, 28 mesh). Gently wash the gelatinous mass of embryos and chaff with a stream of tepid water. Embryos will pass through the sieves and be collected in 14 mesh sieve, some embryos will be found on the lowest sieve (28 mesh).

Wash the contents of the sieves into an enamel photographic tray, or similar size container. A black bottom provides contrast and facilitates observation of the embryos. Remove the embryos from the chaff and other debris. This can be accomplished with a glass tube and rubber bulb. The embryos

can be collected in a container such as a Gooch 4A crucible, pyrex dish with high sides or small kitchen tea strainer. Care should be taken at all times when handling the embryos as they are fragile and will break-up easily. Separated embryos are placed in a flat thick bottom petri dish, covered with 95% ethanol and allowed to stand 15-20 minutes. Non-embryo debris (which turns white) should be removed. A small piece of fine mesh screen can be used to collect the embryos in when draining off the water or ethanol.

5.7 CLEARING OF EMBRYOS

The embryos are removed from the alcohol and placed in a glass petri dish containing 25 mL of 85% lactic acid/trypan blue solution. The dish is covered and allowed to sit overnight at 20-25°C. Embryos must not be left in the lactic acid/dye solution for prolonged periods (over 24 hours) of time as they will begin to soften and shrink making handling and observation difficult. The lactic acid/trypan blue solution is then drained off. A small piece of fine mesh screen can be used to collect the embryos in when draining off the lactic acid/trypan blue solution. The embryos must be placed in a clean petri dish containing sufficient glycerine to cover them and be covered with a lid.

5.8 EXAMINATION

a. General. After clearing, the excess glycerin is drained off. Just a few drops are required to facilitate the reading of the embryos and prevent them from drying out. Too much liquid on the embryos will create a glare which makes examination difficult.

The embryos vary from greyish blue through powder blue to turquoise depending upon the cultivar and variety. The mycelium of *Ustilago nuda* takes up the stain much more readily than the surrounding tissue and appears as a mass of dark blue threads that can be followed through the embryo. At 10X magnification the mycelia can be observed within the embryo and distinguished from wrinkles and damaged areas of the embryo which also stain darkly.

NOTE: Embryos may be preserved in glycerin for long periods of time without deterioration.

b. Examination of Embryos. In order to facilitate examination and counting, arrange the extracted and cleared embryos into four groups each containing at least 100 embryos. Use a stereoscopic 10X-25X magnification with sub-stage illumination. Carefully examine 100 embryos from each of the four groups. During the examination procedure infected embryos must be counted and the total recorded on the worksheet.

c. Signs of Infection. Mycelium 3: thick radiating as if from the plumular area but confined to the scutellum. Infection may vary from a few strands to complete invasion of the scutellum tissues. Occasionally fungi other than *Ustilago nuda* occur in the scutellum but are usually darker and quite distinct.

For confirmation infected embryos can be removed, placed on a microscope slide, 'squashed' and examined under high power microscope. True loose smut hyphae are strongly septate, branched and uneven in diameter.

5.9 CALCULATION OF INFECTION AND REPORTING RESULTS

Results are expressed as a percentage by number. The percent true loose smut in the sample is calculated on the total number of embryos examined and not on the number of seeds soaked.

The percent true loose smut is calculated by counting the number of non infected embryos and the number of infected embryos and calculating the percentage of the infection based on the total number of embryos examined. The result must be reported as a whole number. If the result is a fraction of 0.5% or greater it must be rounded up to the nearest whole number if the result is a fraction of less than 0.5% it must be expressed as “trace”. For example if the result was 2.8% True Loose Smut, this must be rounded to the next whole number and reported as *Ustilago nuda* (True Loose Smut): 3%

embryos infected. A result of 2.4% would be reported as *Ustilago nuda* (True Loose Smut): 2% embryos infected. The infection must be reported using both scientific and common names as follows:

"*Ustilago nuda* (True Loose Smut): [X]% embryos infected"

5.10 CHECKING LIMITS

5.10.1 Checking Limits for True Loose Smut Percentage

The following checking limits are used to decide when a "check" test is required for the percentage determination for True Loose Smut. In order to use this table, the analyst must consult Grade Table II to determine the applicable grade maximum for true loose smut.

Grade Standard for True Loose Smut	Conduct a check test if the percent True Loose Smut falls between	
Maximum Percent	**Lower Limit**	**Upper Limit**
2%	1.52	2.48
4%	3.35	4.65
6%	5.22	6.78

5.10.2 Maximum Tolerated Differences Between Tests

The following table is used to determine if the check test is compatible with the first test. If the two tests are compatible the True Loose Smut test results are averaged. Enter the table at the average of the two percent True Loose Smut tests. If the range between the two tests being compared is equal to or less than the tolerance, the two tests are averaged and this result is reported. If the difference between the original and check test exceeds the tolerance conduct a third test and average the results which are compatible according to the table. If the third test falls between the first two and is compatible with both, then report the average of the three tests.

Average of Two Tests %	Tolerance %
1.75 – 1.99	0.97
2.00 – 2.24	1.02
2.25 – 2.49	1.08
3.00 – 3.49	1.25
3.50 – 3.99	1.33
4.00 – 4.49	1.41
4.50 – 4.99	1.48
5.00 – 5.99	1.59
6.00 – 6.99	1.72

5.11 REFERENCES

International Rules for Seed Testing (ISTA), Seed Health Method: 7-013 available at

http://www.seedtest.org/en/home.html