the NEWS LETTER

Volume 63, No. 2 February, 1989

of the

ASSOCIATION OF OFFICIAL SEED ANALYSTS

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January 12, 1989

To: AOSA Members

From: Stephen J. Hurst, Chairman
AOSA Rules Committee

Subject: Proposed rule change to delete APPENDIX 1. Seedling Descriptions from the AOSA Rules and to adopt the Seedling Evaluation Handbook and reference it in Section 4.5b.(3) of the Rules.

This special issue of the Newsletter is necessary so that a rule proposal submitted by the AOSA Seedling Evaluation Committee can be presented to the AOSA membership. This proposal and associated handbook has been evaluated and approved by the Rules Committee. Please read and review this information carefully. The proposal and the Seedling Evaluation Handbook will be discussed and voted on by the AOSA membership at the June 1989 annual meeting in Illinois. Please bring this issue of the Newsletter to the annual meeting with you. Comments that anyone has concerning this proposal or format and content of the handbook can be forwarded to:

Stephen J. Hurst, Chairman
AOSA Rules Committee
Federal Seed Laboratory
USDA, AMS, Seed Branch
Bldg. 306, Rm. 214, BARC-E
Beltsville, Maryland 20705

Attachments

PROPOSAL

Delete Appendix 1. Seedling Descriptions from the AOSA Rules for Testing Seeds and adopt the Seedling Evaluation Handbook and reference it in Section 4.5b.(3) of the Rules.

PRESENT RULE

APPENDIX 1. Seedling Descriptions (pages 105a - 116 of Rules)

Section 4.5b.(3) Seedling descriptions for specific kinds and groups as set forth in the appendix to the rules. These descriptions are a condensed version of those given in U.S. Department of Agriculture No. 30, "Testing Agricultural and Vegetable Seeds." The only changes in interpretation from the descriptions in Handbook No. 30 pertain to (a) cotyledon evaluation in beans, lettuce, group B-Asteraceae, and group B-Fabaceae and (b) root evaluation in group D-Poaceae. With these exceptions, the seedling descriptions in Handbook No. 30 are to be regarded as a further elaboration of those given in the appendix.

PROPOSED RULE

(1) Delete Appendix 1. in its entirety from the AOSA Rules and replace it with a separate Seedling Evaluation Handbook.
(2) Change Section 4.5b.(3) in the Rules to read:
Seedling descriptions for specific kinds and groups are set forth in the Seedling Evaluation Handbook. This entire handbook shall be considered part of the Rules.

REASONS FOR THE PROPOSED RULE OR CHANGE

Deletion of Appendix 1. from the Rules and placement of seedling descriptions in a separate handbook is considered necessary in order to remove ambiguities, standardize terminology, and provide supplemental information. In a survey of AOSA and SCST laboratories, 95% of the respondents felt that some form of revision to Appendix 1. was required. Section 4.5b.(3) is revised to reflect the deletion of Appendix 1. from the Rules. Also, references to seedling descriptions in Handbook No. 30 are no longer considered necessary and are deleted from this section in the proposal.

SUBMITTED BY AOSA Seedling Evaluation Committee
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PROPOSED

SEEDLING EVALUATION HANDBOOK

Prepared by the

Seedling Evaluation Committee
Association of Official Seed Analysts

Published by the Association

1989

PREFACE

The Seedling Evaluation Handbook is presented here as the first of two parts of a proposal to replace Appendix 1, Seedling Descriptions, of the AOSA Rules for Testing Seeds (1981, revised 1984, 1985, 1986 and 1987). It is to be presented to the AOSA membership for adoption at the annual meeting of the Association in June 1989, at Peoria, Illinois.

The Committee's objectives in preparing this first part were to: a) remove ambiguity from the descriptions; b) standardize the terminology used across the descriptions; c) introduce background and supplementary information; and d) provide the framework for the enhancements of the second part of the project. It was necessary to make many changes in order to meet these objectives; however, every effort was made to avoid introduction of substantive changes to Appendix 1.

The objectives for the second part will be to add: a) any changes which could be considered substantive (e.g. a rewording of the split coleoptile rule for the Poaceae); b) new descriptions for major families now included in the miscellaneous section (e.g. Solanaceae); and c) drawings of normal and abnormal seedlings. These changes and additions should substantially improve the descriptions.

It is hoped that the second part of the proposal will be prepared in time for publication in the February 1990 Newsletter, in preparation for presentation at the June 1990 annual meeting.

AOSA Seedling Evaluation Committee, 1989

ACKNOWLEDGEMENTS

Support in the preparation of this handbook has been provided by the Association of Official Seed Analysts, the Society of Commercial Seed Technologists, Agriculture Canada, the United States Department of Agriculture, and member laboratories of these organizations.

The "Handbook for Seedling Evaluation", 1979, published by the International Seed Testing Association (ISTA) inspired the initiation of this work. Early discussions with Drs. Jan Bekendam and Regula Schmitt-Grob of the ISTA Germination Committee were particularly helpful.

The following members of the AOSA Seedling Evaluation Committee have actively participated in the planning and preparation of this handbook: Doug Ashton (Chairman), Prof. Leroy Everson, Beverley Jackson, Jeff Ruprecht, John Scott, Terry Turner and Coralie Wilson. Earlier members of the Committee included A. B. (Sandy) Ednie, Dr. Wayne Guerke, Oscar Hall and Harry Smith. Many other persons have made substantial contributions to the preparation and review of the descriptions; their suggestions and encouragement have been invaluable.

SEEDLING EVALUATION
HANDBOOK

1 Introduction

Laboratory test results are used as a basis for buying and selling seed, for the labeling of seed as required by federal and state laws, and for monitoring the labeling to ensure it is current and truthful. Since it is essential that accurate test information be provided, all commercial and official seed testing laboratories must follow accepted rules for testing. These rules must be fully explained and standardized.

A major step towards standardization was taken in 1952 with the publication of USDA Handbook 30, "Testing Agricultural and Vegetable Seeds". This book provided valuable information on seed testing, including the first descriptions of normal and abnormal seedlings. In 1960, the Handbook 30 descriptions were modified to form prescriptive statements and appended to the AOSA Rules for Testing Seed. As Appendix 1 to the Rules, the descriptions received "official" status.

While the addition of the seedling descriptions to the Rules was an important step towards uniformity in germination testing, most of the supplementary information included in Handbook 30 was not included. In addition, it had been recognized for some time that some ambiguities and gaps existed in the descriptions. In 1978, therefore, the Association undertook a review of Appendix 1 with the aim of updating and clarifying the descriptions. Many analysts also expressed the wish to have supplemental information and illustrations included which would provide a basis for the understanding of the descriptions.

The objective of this handbook is to further standardize the evaluation of seedlings by providing, in addition to the evaluation rules, a training aid and working reference which includes: botanical information on seeds and seedlings; a glossary of relevant terms; seedling evaluation criteria replacing Appendix 1 of the Rules; information on factors affecting the appearance of seedlings; and drawings of normal and abnormal seedlings.

2 Classification, Structure and Development

Botanical sciences basic to the understanding of seeds and seedlings are: taxonomy, the study of the classification of plants; morphology, the study of the internal and external structure of plants; and physiology, the study of the life processes of plants. Information from these fields of botany provides the seed analyst with knowledge important to the understanding of their work.

2.1 The Classification of Plants

2.1.1 Scientific names

In referring to a plant, either its common name or its scientific name may be used. The plant's scientific name consists of a binomial composed of the genus name (e.g. Medicago) and the species name (e.g. sativa). Further subdivisions such as subspecies or variety may exist. The scientific name provides information on the family relationship of the plant which often is useful. For example, you would know that Medicago sativa and Medicago lupulina are closely related, a fact which you probably would not deduce from the common names alfalfa and black medick. In addition, scientific names are essentially standardized throughout the world, while common names vary from region to region, often resulting in confusion. For example, Medicago sativa is known as alfalfa in the United States and Canada but as lucerne in many other countries. The seed analyst is advised to learn the scientific names of the species which are encountered in the laboratory.

Family names usually end in "-aceae"; however, there are eight families, named before standardization of botanical nomenclature, for which two names exist. While the older, traditional names are still valid, the AOSA has elected to adopt the form with the standardized "-aceae" endings. The eight families and their synonyms are as follows:

Apiaceae = Umbelliferae
Arecaceae = Palmae
Asteraceae = Compositae
Brassicaceae = Cruciferae
Clusiaceae = Guttiferae

Fabaceae = Leguminosae
Lamiaceae = Labiatae
Poaceae = Gramineae

2.1.2 Taxonomy

Taxonomy is the science of classification dealing with the arrangement of plants and animals into categories according to their natural relationships. The following classification of plants is commonly used: (1) bacteria; (2) algae; (3) fungi; (4) mosses and liverworts; (5) ferns; and (6) seed bearing plants. This last category may be further divided into the gymnosperms (which include, for example, the conifers), and the angiosperms, or flowering plants. In this handbook, only the angiosperms will be considered.

The angiosperms are divided into two sub-classes: dicotyledons (possessing two cotyledons), and monocotyledons (possessing one cotyledon). The major agricultural and vegetable crops are members of two families of the monocotyledons and about 15 families of the dicotyledons.

The two most important monocotyledonous crop families are the Poaceae, with corn, cereal crops and lawn and pasture grasses being examples, and the Liliaceae, which includes asparagus and onion. The remainder of our agricultural and vegetable crops are dicotyledonous. Three examples are: Fabaceae, including beans, clovers and peas; Asteraceae, with the main crops being sunflower and lettuce; and Cucurbitaceae which includes cucumbers, pumpkins and squash.

2.2 Seed Development

2.2.1 The ovule

A seed is a mature fertilized ovule containing an embryonic plant; usually it has nutrient storage tissue and is surrounded by a protective coat, the testa. Differentiation of a cell in the wall of the ovary initiates the development of an ovule. Each ovule begins as a dome shaped swelling referred to as the nucellus. One or two ring-like layers of tissue form around the base of the nucellus. These layers, the integuments, grow until they surround the entire nucellus, except for a tiny pore where they come together. The pore is known as the micropyle, while the collective term used for the integuments is "testa" (seed coat). The stalk connecting the ovule to the ovary wall is called the funiculus, with its place of attachment to the integuments being termed the hilum. Inside the ovule, the embryo sac develops from a specialized cell of the nucellus called the megaspore.

2.2.2 Fertilization

For every ovule that later becomes fertilized, a pollen grain must germinate on the stigma of the pistil and grow down the style to the embryo sac. As the pollen tube enters the embryo sac it disintegrates and the contents are discharged. Each of two specialized cells from the pollen tube fuses with cells of the embryo sac. One of these fusions results in the development of the embryo, while the other results in the development of the endosperm tissue. These two fusions are referred to as "double fertilization".

2.2.3 Storage tissue

The storage tissue in seeds may originate from three sources depending on the species:

a Perisperm

The nucellus may persist as the storage tissue, in which case it is referred to as "perisperm". In Beta vulgaris (beet) the storage tissue is perisperm and there is little or no development of the endosperm following fertilization.

b Endosperm

Endosperm is one of the products of double fertilization, and in some species, particularly those of the Poaceae, it develops as the storage tissue. In this event, little or no nucellus tissue remains, and the extent of cotyledon development varies.

c Cotyledons

In some species the embryo continues to grow until the endosperm is absorbed and replaced by the enlarging cotyledons (e.g. Phaseolus vulgaris). For other species or genera, the perisperm and/or endosperm may persist in varying degrees together with the cotyledons.

2.2.4 The embryo

The product of one of the fusions of the fertilization process is the embryo (the other being the endosperm). Depending on the species, the embryo develops to varying degrees within the seed, becoming a "miniature plant" by the end of the growing season. In Phaseolus vulgaris, for example, the embryo is fully developed and the radicle, hypocotyl and epicotyl with primary leaves can easily be observed. The development of the embryo in other species may be much less, with some essential structures being observed only after considerable growth of the seedling.

2.3 Definition of the Seed

Botanically, a seed is a mature fertilized ovule containing an embryonic plant; usually it has nutrient storage tissue and is surrounded by a protective coat, the testa. This structure is a "true seed"; however, the ovules of many species have additional structures of the mother plant attached or fused to the seed coat. For example, the "seed" of Triticum aestivum (wheat) is botanically a fruit because the pericarp (ovary wall) is fused with the seed coat. In this handbook the term "seed" will be used in the agronomic sense (i.e. the "true seed" plus any accessory structures which may be attached when it is planted in the field).

2.4 Seedling Structure

2.4.1 Dicotyledons

a The root

The root system serves three major functions: (1) to anchor the plant in the soil, (2) to absorb water and dissolved salts from the soil and (3) to conduct the water and salts to the hypocotyl, cotyledons and epicotyl.

The embryonic root, or radicle, is located at the basal end of the embryo and is usually the first seedling structure to rupture the testa. After emergence it is referred to as the primary root. The primary root elongates rapidly and soon numerous root hairs develop, greatly increasing the absorbing surface of the roots. As the seedling continues to grow, secondary roots develop from the primary root and from other secondary roots. Roots may also emerge from other structures (e.g. the hypocotyl) and are referred to as adventitious roots.

b The hypocotyl

The portion of the seedling axis between the root and the cotyledons is the hypocotyl. The hypocotyl is a transition structure for the transport of water and dissolved salts from the roots to the epicotyl. When a seed with epigeal germination (see section 2.5 for definitions of epigeal and hypogeal germination) is planted in moist soil, the hypocotyl elongates carrying the cotyledons above the soil surface.

c The Cotyledons

The cotyledons are the storage structures of the embryo. They may be only a small portion of the seed in species with endosperm or perisperm storage tissue, or they may occupy a large portion of the embryo when they are the primary storage tissue (e.g. Phaseolus vulgaris). In epigeal species, the cotyledons may grow quite large and become the first photosynthetic structures of the young plant. In hypogeal species the primary function of the cotyledons is to provide nutrients to the growing seedling until it can produce its own nutrients. In most species the cotyledons shrivel and drop off as their reserves are depleted. In a few species (e.g. Cucurbita pepo, pumpkin) the cotyledons may persist well beyond the seedling stage of growth.

d The Epicotyl

The epicotyl includes all seedling structures above the cotyledons. In species with epigeal germination (e.g. *Phaseolus vulgaris*), the epicotyl, cotyledons and part of the hypocotyl emerge from the soil. In species with hypogeal germination (e.g. *Pisum sativum*), only the epicotyl emerges, carrying the first foliage leaves above the soil surface. In these species, the epicotyl also bears one or more scale leaves. Dormant meristematic buds in the axils of these scale leaves become active if there is damage to the terminal bud. The conducting tissue of the epicotyl transfers water and nutrients from the hypocotyl and cotyledons to the leaves and terminal bud above.

e The Terminal Bud

The terminal bud occupies the tip of the epicotyl and consists of the apical meristem surrounded and protected by the developing leaves.

2.4.2 Monocotyledons

a The Root

As in the dicotyledons, the monocotyledon root system serves to anchor the plant in soil, absorb water and dissolved salts from the soil and to conduct the water and salts to the growing seedling.

The embryonic root, or radicle, is situated at the basal end of the embryo and, in the case of the Poaceae, its apex is covered by the coleorhiza. After the radicle emerges it is referred to as the primary root. In some species of the Poaceae (e.g. *Triticum*) the primary root is indistinguishable from the other roots which develop from the scutellar node region and hence all of these are referred to as seminal roots. Roots that develop from structures above the scutellar or cotyledonary node are called adventitious roots. Secondary roots may develop from seminal and adventitious roots.

b The Hypocotyl and Mesocotyl

In monocotyledons the hypocotyl is usually not discernible as a separate structure. The mesocotyl is the part of the seedling axis between the scutellum and the base of the coleoptile. In some species (e.g. *Zea mays*) the elongation of the mesocotyl may be considerable. In others (e.g. *Triticum aestivum*) the elongation may be imperceptible. Elongation of the mesocotyl is suppressed by light after the coleoptile emerges from the soil.

c The Cotyledon

In the monocotyledons the cotyledon absorbs nutrients from the endosperm and transfers them to the growing seedling. In the Poaceae the cotyledon is called the scutellum. It is in close proximity to the endosperm and is laterally attached to the embryo axis. In *Allium* (Liliaceae) the cotyledon tip remains embedded in the endosperm to absorb nutrients but the cotyledon also emerges from the soil (i.e. germination is epigeal) and becomes photosynthetic.

d The coleoptile

The coleoptile is only present in species of the Poaceae. It is a leaf-like, cylindrical sheath enclosing the terminal bud of the embryo and the developing leaves of the young seedling. The coleoptile provides protection for the leaves as they push up through the soil. After emergence from the soil, growth of the leaves ordinarily causes the coleoptile to split downward from the tip. The coleoptile does not persist beyond the seedling stage.

2.5 Seedling Growth Habit

2.5.1 Epigeal germination

Epigeal means "above the earth", referring to the cotyledons which are raised above the soil surface after the seed germinates. In the dicotyledons the hypocotyl forms an arch and, as it elongates, the cotyledons with the terminal bud enclosed are pulled above the soil surface. The hypocotyl straightens as it continues to elongate. In Allium cepa the cotyledon itself elongates and emerges from the soil. In this case there is little or no significant hypocotyl elongation.

2.5.2 Hypogeal germination

Hypogeal means "below the earth", in reference to the position of the cotyledons. In hypogeal species there is little or no hypocotyl elongation; the cotyledons remain below the soil surface and the primary leaves become the first photosynthetic structures, rather than the cotyledons. In some species (e.g. Pisum sativum) the epicotyl elongates and pushes the terminal bud above the soil surface. In many species of the Poaceae (e.g. Zea mays) the mesocotyl elongates bringing the terminal bud to, or just below, the surface.

2.6 The Germination Process

When moisture is supplied to dry seeds water enters by imbibition, resulting in swelling of the embryo tissues. During imbibition the seed coat softens and becomes more permeable to water and gas. Cellular tissue is hydrated and the germination process begins if conditions of temperature, gas concentration and light are favorable. Enzymes are activated, starch is converted into sugar, fats into soluble compounds and proteins into amino acids. Nutrients are transferred from the storage tissues (endosperm, perisperm or cotyledons) to the root/shoot axis of the embryo. Growth may begin by cell elongation or by cell division and the radicle emerges from the seed. The hypocotyl or epicotyl elongates and the seedling emerges from the soil.

3 Seedling Evaluation

To determine the percentage germination of a sample it is necessary to evaluate the essential structures of the seedling. In order to establish uniformity between laboratories in this assessment, seedling descriptions have been developed and form section 4 of this Handbook. There are many factors which interact to affect the appearance of seedlings in the laboratory. The analyst must have an understanding of these factors to evaluate the potential of the seedling to continue development.

3.1 Laboratory Definition of Germination

In seed laboratory practice, germination is the emergence and development of the essential structures of the seed embryo which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favorable conditions. The objective of the germination test is to determine the percentage of normal seedlings in the test.

3.2 Germination vs. Vigor

Germination test results correlate well with emergence under favorable field conditions. The germination test is not intended to consider the vigor of the seed. Since the vigor of a seed lot refers to its ability to produce seedlings under diverse conditions (e.g. cold or hot weather, wet or dry soil, varying storage periods, etc.) it is evident that a vigor test must be planned to determine the performance of a seed lot under specific environmental conditions. The laboratory germination test is not designed to provide vigor information. Rather, its purpose is to establish the maximum plant-producing potential of a seed lot. Vigor testing methods are described in the AOSA Seed Vigor Testing Handbook.

3.3 Assessment of Seedlings

3.3.1 Normal seedlings

In general, seedlings are classified as normal if they have no defects or only slight defects which will not impair the continued development of the seedling or plant when grown in soil under favorable conditions.

3.3.2 Abnormal seedlings

Seedlings are classified as abnormal if they have defects which will prevent them from developing into mature plants when grown in soil under favorable conditions. These defects are not to be considered abnormalities if they are caused by test conditions. To classify a seedling as abnormal the analyst must judge the defect to be so severe that further development of the seedling would be unlikely. Specific abnormalities are listed in the seedling descriptions of Section 4.

3.4 Causes of Abnormal Seedlings

There are many causes of seedling abnormalities. If seedlings are difficult to assess or the analyst suspects that the defects may be exaggerated on the "artificial" germination substrata (particularly if chemical injury or the presence of disease are involved), it is advisable to retest the seed in sand, soil or a sand/soil mixture.

3.4.1 Mineral deficiencies in the soil

Crops grown on soils with mineral deficiencies may produce seeds which produce abnormal seedlings when germinated. The abnormals are usually characterized by shrunken, hollow, brown or pithy areas on the cotyledons. They may also have decayed areas on the cotyledons, hypocotyls, epicotyls or roots and may be stunted and undeveloped.

A calcium nitrate solution may be used for overcoming hypocotyl collar rot of bean seedlings, but chemical treatments have not yet been authorized for other types of mineral deficiencies. The analyst should learn to recognize mineral deficiencies as discussed under the various family groups.

3.4.2 Frost damage

Freezing temperatures when seeds on the plants are in the developmental stage can cause damage to the seed. The degree of damage depends on the species, the stage of seed development and severity of the frost. Germination and growth may be initiated in frost-damaged seeds but the resultant seedlings are often too weak to produce normal plants. Seedlings from frost-damaged seeds of the Poaceae may be characterized by grainy coleoptiles and spirally twisted leaves as well as decay at the point of attachment to the scutellum.

3.4.3 Heating

Over-heating can occur if seed is too moist when the crop is harvested and it is not given an opportunity to dry before further storage. Fungal activity plays a major part in temperature increase in moist seed. Heated seed often shows a high percentage of dead and moldy seeds, or seedlings which decay after sprouting. Seedlings may have missing roots or epicotyls or, in the Poaceae, missing, stunted or empty coleoptiles. Heating may also result in decay at the point of attachment to the scutellum. Damage may range from minor to severe and seedlings may be difficult to evaluate.

3.4.4 Mechanical damage

Mechanical breakage of seeds may occur during harvesting, threshing, loading, hauling, unloading, and cleaning operations. Mechanical damage to grass seed and especially to some of the range grasses may occur during combining or in special milling processes designed to remove weeds or accessory seed structures. Large-seeded legumes such as field and garden beans, lima beans, soybeans and peas 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. Other legume seeds such as the larger seeded clovers and vetches may be damaged to a lesser extent in threshing. Some damage may occur during scarification intended to reduce the hard seed content in legume seeds. Damage at the point of attachment of the cotyledons may be difficult to evaluate if seedlings are removed too early in the test period.

3.4.5 Insect damage

Seeds that have become infested with insects may produce seedlings which lack essential seedling structures or are weak and stunted. In some cases the adult insect lays her egg in the developing ovule and the damage is caused by the larvae eating away the tissues inside the seed coat. Examples of these include weevil damage to seed of field peas, cowpeas and vetch, and chalcid fly damage to alfalfa and red clover seed. Some storage insects eat away the embryo and scutellum and leave the endosperm, in which case the seed will not germinate. Other insects eat the endosperm and leave the embryo, which may germinate but be too weak to continue development.

3.4.6 Chemical treatment injury

Some fungicides or insecticides, used for seed treatment, can cause abnormal seedlings in germination if excess amounts of the chemicals are used. When seeds have been "over-treated" and planted on "artificial" substrata the seedling roots and hypocotyls may become stunted and swollen. In severe cases, some essential seedling structures may be destroyed.

When insecticides or herbicides are used in the field they may affect the seed produced, particularly if treatment occurs at the early stage of seed development. A more common cause of insecticide or herbicide damage is storage of seed near these chemicals in a warehouse. Seed mailed in "empty" herbicide bags or boxes have been observed to produce abnormal seedlings in the germination test.

Retesting in sand or soil is recommended when damage due to chemical exposure is suspected.

3.4.7 Declining vigor

Seeds which are old, or have been subjected to unfavorable storage conditions are usually slow to germinate. Seedlings may be weak and watery in appearance. Some of the essential structures may be stunted or lacking and saprophytic fungi may interfere with growth.

3.4.8 Pathogenic infections

Although seed infected with pathogenic organisms may initiate growth, one or more of the essential seedling structures may be damaged or destroyed by fungi or bacteria. Since the manifestation of disease on the seedlings is dependent on the environmental conditions during germination, test results may be erratic. When the seedlings of a test are badly infected with pathogenic organisms, the germination analyst must be careful in differentiating between primary and secondary infections. Retests in sand or soil are recommended when evaluation of seedlings is difficult. See also section 3.5.5, diseased and decayed seedlings.

3.4.9 Toxicity in the laboratory

If seed is planted on substrata placed directly on galvanized trays, or galvanized trays coated with a thin copper finish, the seedlings may show zinc toxicity. The most common symptom of zinc toxicity is stunted, thickened and discolored roots. If galvanized trays must be used, they should be covered with plastic or waxed paper or the seeds should be planted in a container that can be placed on the tray.

Artificial substrata such as towelling, blotters and creped cellulose may contain chemicals toxic to seedlings. Laboratories should purchase only artificial substrata produced for seed testing. Paper towels intended for the washroom and creped cellulose produced for packaging are not suitable. Sulfuric acid not thoroughly rinsed from the paper pulp, sizing sometimes used to give paper a hard surface or binders intended to hold paper together may be sources of the toxicity.

Tap water containing toxic chemicals may cause germination failure, root inhibition or other seedling abnormalities. Distilled water should not be beyond suspicion; it could have a low pH which may adversely affect germinating seeds.

Germination substrata or water from new or unknown sources should be tested for phytotoxicity prior to routine use. Plant seeds of sensitive species (e.g. timothy, lettuce, celery or sorghum) on the substrate to be tested as well as on a similar substrate known to be non-phytotoxic. Stunted roots or hypocotyls, or roots which arch away from the substrate are signs of phytotoxicity. A comparison of the test and control samples should be made daily, because the symptoms may be more difficult to see once the roots become entangled.

3.5 Factors Affecting Seedling Evaluation

3.5.1 Timing of the seedling evaluation

a Preliminary or first count

One or more preliminary counts may be required before the end of the prescribed germination period for some species. Interim counts are desirable so entanglement of the seedlings does not cause an evaluation problem at the time of the final count. The number of days given in the germination tables for the first count is only approximate, with a deviation of one to three days being permitted. Only seedlings that are sufficiently developed to be assessed as normal should be removed at the first or interim counts. Seedlings with incomplete development should be left for the full prescribed period. Badly decayed seedlings and moldy seeds should be counted and removed at the first or interim counts to avoid the spread of infection to other seeds and seedlings.

b Final Count

The germination percentage is based on the evaluation of essential seedling structures at the preliminary and final counts. For epigeal species it is recommended that the cotyledons be free of the seed coat at the time of the count. However, the degree of development of seedling structures at the final count varies by species and seed lot. In some species (e.g. Phaseolus vulgaris) the essential structures are well formed in the embryonic stage and are usually well developed and clearly visible at the time of the final count. In these species, if the seed coat has not been shed, it should be removed and the cotyledons and terminal bud examined to determine if they are healthy. In other species (e.g. Daucus carota, Trifolium pratense) the essential structures develop slowly and may not be fully developed at the time of the final count. In these species, the seedling descriptions indicate that presence of the structure may be assumed if the surrounding tissues are intact.

Tightly adhering seed coats may be an indication that the cotyledons are necrotic or decayed; such coats should be removed and the cotyledons examined.

If at the end of the prescribed test period, the seedlings are not sufficiently developed for positive evaluation, the test may be extended two more days for the species of most families. Tests may be extended five additional days for species of Convolvulaceae, Geraniaceae, Malvaceae and Fabaceae.

3.5.2 Seedling response to moisture, light and temperature.

a Moisture

An adequate supply of water and oxygen is essential for seed germination and seedling development. Water enters seeds by imbibition, resulting in hydration of the embryo tissues and, if conditions are suitable, activation of the hormonal and enzymatic systems which initiate the germination process.

As stated in the AOSA Rules, the substratum must be moist enough to supply the needed moisture to the seeds at all times. However, the following caution is included: "Avoid supplying excessive moisture which will restrict aeration of the seeds. Except as provided for those kinds of seeds requiring high moisture levels in the germination media, the substrata should never be so wet that a film of water is formed around the seeds." Paper substrata should not be so wet that, by pressing, a film of water is formed around the finger, although for the larger seeds slightly more moisture may be required. For further information on the moisture requirements refer to section 4.3 of the AOSA Rules. Also, for special moisture requirements for the seeds of certain species (e.g. Beta vulgaris, Oryza sativa, etc.) refer to section 4.8 of the Rules (special procedures and alternate methods).

When the methods prescribe "moisture on dry side", the moistened substratum should be pressed against a dry absorbent surface such as a dry paper towel or blotter to remove excess moisture.

When sand or soil is used as the substratum, water should be added until the consistency of the sand or soil is such that when formed into a ball in the hand, it is easily broken when squeezed between two fingers. After moistening it should be put into containers for the test without packing.

When closed plastic containers are used for germination tests, it is seldom necessary to add water subsequent to planting, particularly if the test is of short duration. However, the germination analyst should check substrate moisture at regular intervals, regardless of test duration, to make certain that the moisture content is optimum.

b Temperature

The effect of temperature on germinating seeds is often expressed as cardinal temperatures (i.e. the minimum, optimum and maximum temperatures) at which seeds of a species will germinate. The optimum temperature -- that temperature at which the greatest percentage of germination occurs within the shortest time -- is the temperature prescribed in the Rules.

The response of seed to germination temperature depends on the species, variety, region of production and the length of time from harvest to the date the test is conducted. In general, seeds produced in temperate regions require lower germinating temperatures than seeds from a tropical region. Seeds of wild species generally have lower temperature requirements than those from plants long domesticated.

Seeds of many species require a daily alternating temperature for optimum germination. The seeds of species grown in temperate or northern climates are more likely to respond to alternating temperatures than those grown in warmer climates. Ordinarily when alternating temperatures are specified in the Rules, the seed is kept at the low temperature for 16 hours and at the high temperature for eight hours. The seeds of some grass species will germinate well with a very wide alternation of temperature (e.g. 10-30°C).

Some kinds of seed need to be prechilled to break dormancy, particularly if testing is to be done soon after harvest. Generally, these seeds lose their dormancy with time, but in some species the dormancy may be very long-lived (e.g. some of the native range grasses). To prechill a sample, the seed is planted in or on the moist substrate and placed at a low temperature (usually 5 or 10°C) for a few days. The temperature and duration of the prechill are prescribed by the Rules. At the end of the prechilling period, the sample is transferred to the normal testing temperature.

Temperature is an important factor in seed testing standardization. If a number of laboratories germinate the same seed sample or lot, and expect to obtain comparable test results, it is important that they use a temperature specified in the AOSA planting prescriptions. Daily checks should be made to ensure that the temperature prescribed in the Rules is maintained and is as uniform as possible throughout the germinator. Care should be taken to avoid temperature "over-run" for tests under, or near, the light source. "Hot spots" should be identified and eliminated wherever possible.

c Light

The seeds of some plant species are light sensitive (with germination being either stimulated or inhibited), while others are not. Light sensitivity during seed germination depends on the species and variety as well as environmental factors that have affected the seed before and during germination. Seeds of many of the species of grasses and some of the vegetables require light to obtain complete germination. In general, freshly harvested seeds are more sensitive to light than "aged" seeds.

Both light intensity (candle-power) and light quality (color or wave length) influence germination. The Rules specify a light intensity of 75-125 foot candles (i.e. 750-1250 lux) for the germination of dormant seeds.

Where prescribed in the Rules, light should be provided by a cool white fluorescent source, for at least eight hours in every 24. Those species requiring light should be germinated on the top of the substratum. When an alternation of temperature is used, seeds should be illuminated during the high temperature period. Although light is prescribed for some species, no distinction is made between dormant and non-dormant seeds within these species. Illumination of non-dormant seeds is generally beneficial (except for those inhibited by light) since the seedlings become green and the essential structures are easier to evaluate. One caution is needed since lights produce heat: the germination analyst should make certain the temperature at the substratum level never exceeds the prescribed temperature.

3.5.3 Counting seedlings of multiple seed units and coated seeds

Seed units containing more than one true seed, e.g. beet clusters (Beta vulgaris), New Zealand spinach (Tetragonia tetragonioides) or multiple florets of certain grasses (e.g. Dactylis glomerata), are tested as single seeds and are classed as normal if at least one seedling develops and continues to grow under favorable conditions. When a seed unit (i.e. a single cluster or multiple floret) produces two or more seedlings it is only counted as one seedling.

For coated seeds the seed units should be placed on the substratum in the condition in which they are received. No rinsing, soaking or other pre-treatement should be used. Each coated seed is considered a seed unit when counting the seedlings. If symptoms of phytotoxicity are evident on seedlings from coated seeds planted on artifical substrata, a retest should be conducted in sand or soil.

If coated seed is received with a request for a test on de-coated seed, then the germination report should include specific information about the procedure used.

3.5.4 Hard, swollen, dormant and dead seeds

Hard seeds are seeds which remain hard at the end of the prescribed test period because they have not absorbed water due to an impermeable seed coat. Species known to produce hard seeds are indicated by footnotes in Tables 3, 4, and 5 of the Rules. The percentage of hard seeds occurring in the germination test will vary with the age, kind, variety and moisture content of the seed. The hard seed content of some freshly harvested legumes such as red clover, lespedeza and field peas may decrease rapidly within the first few weeks or months of dry laboratory storage. Conversely, seeds of okra, vetch and certain other legumes may increase in hard seed content during dry laboratory storage. The hard seededness in beans is increased as the beans become dessicated. The relative humidity of the air in the storage area may cause moisture changes within the seeds and hence changes in the number of hard seeds. These changes are reversible. In reporting the test results, the percentage of hard seeds is reported in addition to the percentage germination.

Swollen seeds are seeds that have imbibed water but have not germinated; they may or may not be visibly larger than seeds that have not imbibed water. Swollen seeds may or may not be viable. They may be observed in germination tests of some species of the Fabaceae, Convolvulaceae, Geraniaceae and Malvaceae. Often a somewhat lower germinator temperature will result in fewer swollen seeds and a higher germination percentage. If there is a question whether seeds are swollen or hard, slight tweezer pressure on the questionable seeds will provide the answer. Care should be used in pressing to avoid damage to swollen seeds. If at the end of the regular germination period swollen seeds remain, all seeds and seedlings, except the seeds that are swollen, should be removed and the test extended five more days. After five days any additional normal seedlings should be included in the percentage germination. Further information concerning procedures related to swollen and hard seeds is provided in sections 4.8, 4.9d(6) and 4.9k(6) of the Rules.

Dormant seeds are viable seeds which fail to germinate when provided with suitable germination conditions as specified in tables 3, 4 and 5 of the Rules. Viability of these ungerminated seeds may be determined by any appropriate method given in section 4.9k of the Rules. The percentage of dormant seeds may be reported in addition to the percentage germination. Additional procedures for the dormant seeds of certain species are given in section 4.8 of the Rules.

All samples suspected of being dormant should be tested under conditions suitable for breaking dormancy, as described in sections 4.8 and 4.9k of the Rules. However, even when appropriate procedures are used, the germination analyst must sometimes distinguish between hard, dormant and dead seeds. Often if there are dead seeds in a sample they become evident after a few days in test. Dead seeds usually begin to decay, become soft, discolored and covered by fungi and bacteria. Dead seeds should be removed from the test to avoid further contamination of the substratum, seedlings and other seeds. Dormant seeds usually remain relatively free of fungi; however, dormant grass florets often become covered by mold and therefore the presence of fungi alone should not be considered as evidence that florets are non-viable.

3.5.5 Diseased and decayed seedlings

Primary infection originates from the seed or seedling itself. Seedlings with slight primary infection are considered normal provided development of none of the essential structures has been impaired. Seedlings with primary infection sufficient to impede development of one or more essential structures are to be classified as abnormal.

Secondary infection is infection which does not originate with the seed itself, but rather from other diseased seeds, seedlings or adhering structures (such as the cluster of Beta). Decay caused by the spread of infection from the surface of adhering seed coats should be considered secondary. Seedlings with any degree of secondary infection are to be classified as normal provided that all essential structures are present and otherwise normal.

When decay is present in a test, counts should be made at approximately 2-day intervals between the first and final counts, with obviously dead and moldy seeds being removed and recorded at each count. Samples should be retested if infection is extensive enough to make accurate evaluation difficult, or if improper test conditions may have contributed to the development of the infection.

Practices to minimize spread of mold include wider spacing of seeds, proper temperature control, removal of decayed seeds, adequate aeration and keeping the tests as dry as possible while providing adequate moisture for germination. Retesting in sand or soil will usually reduce the level of secondary infection.

3.5.6 Negative geotropism

Negative geotropism is caused by a physiological disorder usually characterized by root structures that grow upward. Seedlings with negative geotropism must be classified as abnormal. However, the germination analyst must make certain that the condition is not caused by poor laboratory conditions. "Apparent" negative geotropism may occur with artificial substrata if adverse moisture conditions are present or if the substrata contain phytotoxic substances. Also, if seeds are planted in tightly packed soil or if the soil surface becomes dry, seedlings may appear to have negative geotropism. If test conditions are suspected to be the cause of negative geotropism, the sample should be retested under favorable conditions, including retests made in sand or soil.

3.5.7 Use of sand or soil

Sand, soil or a sand/soil mixture should be used in a retest whenever difficulty is experienced in judging essential seedling structures. These media provide the following advantages over artifical substrata:

a. Seedlings develop in an environment resembling field conditions. The seedlings appear more natural, therefore the analyst is more likely to correctly evaluate seedlings.

b. Sand and soil are less favorable environments for the growth of saprophytic fungi and bacteria which often proliferate unchecked on artificial substrata. Also, these organisms may be controlled by competition with fungi and bacteria which occur naturally in the soil (if it is non-sterile).

c. Absorption of phytotoxic substances by the soil will often reduce the severity of chemical seed treatment. The soil may also neutralize natural seed inhibitors.

d. The ability of roots to anchor seedlings in sand or soil makes it possible to grow seedlings to a later stage of development than on artifical substrata; this permits observation of the continued development of questionable seedlings.

Germination analysts should become familiar with the appearance of seedlings of all species when grown in sand or soil so they can evaluate seedlings correctly when they are grown on artifical substrata. Simultaneous tests on artificial substrata and in sand or soil are particularly helpful.

3.5.8 The fifty percent rule

Unless otherwise stated in the seedling descriptions, seedlings are to be classified as abnormal if less than 50% of their cotyledonary tissue remains attached or free of decay. Notable exceptions to this rule are identified in section 4.6.1 (some of the large-seeded epigeal Fabaceae), for which the cotyledons may be missing provided the seedling is otherwise normal and vigorous.

4 Seedling Descriptions

In general, the following are considered to be 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 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 should be retested under more favorable 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.

4.1 Aizoaceae, Carpetweed family

4.1.1 Aizoaceae - All kinds

Tetragonia tetragonioides, New Zealand spinach

a General description

Seed unit: Fruit, with enclosing calyx, containing one to many seeds arranged in a ring around its top. Dicotyledonous.

Germination habit: Epigeal.

Food reserves: Leaflike cotyledons and perisperm.

Shoot system: The hypocotyl elongates carrying the cotyledons above the soil 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.

b 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

c **Remarks**

New Zealand spinach is frequently dormant. Removal of the pulp from around the ends of individual seeds in each fruit will cause prompt germination. Care must be exercised in removing the pulp from the large end of the fruit, where the seeds lie close to the surface. It is recommended that magnification be used during this procedure.

4.2 Asteraceae, Sunflower family

4.2.1 Asteraceae - Lettuce

Lactuca sativa, lettuce

a General description

Seed unit: Achene, dicotyledonous.

Germination habit: Epigeal.

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 soil surface. The epicotyl usually does not show any development within the test period.

Root system: A long primary root.

b 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)
- -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 discolored
- -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

c Substrate effects

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

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

d Remarks

Remove attached seed coats for seedling evaluation.

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

One type of necrosis on lettuce cotyledons is a physiological breakdown of the plant tissues, the cause of which has not been determined. It is manifested by discolored areas on the cotyledons, first appearing on or adjacent to the midrib and lateral veins, and should not be confused with the natural pigmentation of the different lettuce cultivars.

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.

4.2.2 Asteraceae - Other than lettuce

Arctium lappa, great burdock
Artemesia ludoviciana, Louisiana sagewort
Carthamus tinctorius, safflower
Cichorium endivia, endive
Cichorium intybus, chicory
Cynara carduncuIus, cardoon
Cynara scolymus, artichoke
Helianthus annuus, sunflower
Taraxacum officinale, dandelion
Tragopogon porrifolius, salsify

a General description

Seed unit: Achene, dicotyledonous.

Germination habit: Epigeal.

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

Shoot system: The hypocotyl elongates and carries the cotyledons above the soil 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.

b 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
-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

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

c Substrate effects

Dry test media may result in slow or abnormal development, bound roots, retarded secondary root development or unshed seed coats.

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

d Remarks

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 4.9d(4) of the Rules.

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

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 tests since the secondary root development is faster than in artificial media.

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

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

For dormant samples of endive, add about 1/8 inch of water at the beginning of the test and remove excess water after 24 hours.

4.3 Brassicaceae, Mustard family

4.3.1 Brassicaceae - All kinds

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

a General description

Seed unit: True seed, dicotyledonous

Germination habit: Epigeal

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 soil surface; the epicotyl usually does not show any development within the test period.

Root system: A long primary root.

b 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

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

Seedling

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

c Remarks

Secondary roots will not compensate for a defective primary root.

4.4 Chenopodiaceae, Goosefoot family

4.4.1 Chenopodiaceae - All kinds

Beta vulgaris, beet, sugar beet, mangel, Swiss chard
Kochia prostrata, forage kochia
Spinacea oleracea, spinach

a General description

Seed unit: Beta monogerm, Kochia and Spinacea: Achene. Beta multigerm: Cluster formed by the thickened, corky, fused flower parts (calyces) enclosing one to several seeds.
Dicotyledonous.

Germination habit: Epigeal.

Food reserves: Leaf-like cotyledons and perisperm.

Shoot system: The hypocotyl elongates carrying the cotyledons above the soil 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.

b 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

c Remarks

See Rules 4.8.c 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.

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

Frequent counts should 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.

4.5 Cucurbitaceae, Curcurbit family

4.5.1 Cucurbitaceae - All kinds

Citrullus lanatus var. citroides, citron
Citrullus lanatus var. lanatus, watermelon
Cucumis melo, muskmelon or cantaloupe
Cucumis sativus, cucumber
Cucurbita spp., pumpkin and squash

a General description

Seed unit: True seed, dicotyledonous

Germination habit: Epigeal

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.

b 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 (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

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

c Substrate effects

Substrata with excessive moisture have been shown to break dormancy in some cases and to induce dormancy in other cases. 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.

d Remarks

Samples should be retested in sand or soil 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.

4.6 Fabaceae, Legume family

4.6.1 Fabaceae - Large-seeded, epigeal (except soybean, peanut, lupine)

Phaseolus vulgaris, garden bean and field bean
Phaseolus lunatus, Lima bean
Vigna radiata, mung bean
Vigna unguiculata subsp. *sesquipedalis*, yard-long 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).

a General description

Seed unit: True seed, dicotyledonous.

Germination habit: Epigeal.

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 soil 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.

b 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 to be assessed unless the seedling is generally weak, in which case the description for garden bean is to be applied to determine if the seedling is 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

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 or adventitious roots

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

c Substrate effects

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, and transverse cracking of the cotyledons.

Seeds of beans should 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.

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 discolored, 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 the sample involved may be retested using a 0.3 to 0.6 percent calcium nitrate solution to presoak the medium (Rules 4.8j).

d Remarks

The percentage of hard seeds is to be reported in addition to the percentage germination (Rules 4.2.d)

If, at the end of the germination period, there are swollen seeds present, or seeds which have just started to germinate, remove everything but these seeds and continue the test. After five additional days, add any normal seedlings to the previous count. (Rules 4.9.d(6) and 4.9.k(6))

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

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

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 or sand, will aid in interpretation of such seedlings. For general instructions on evaluating seedlings infected with fungi or bacteria, see 3.5.5 of this handbook.

4.6.2 Fabaceae - Large-seeded, hypogeal

Cicer arietinum, chickpea
Lathyrus hirsutus, rough pea
Lathyrus sylvestris, flat pea
Lens culinaris, lentil
Mucuna pruriens var. *utilis*, velvet bean
Phaseolus coccineus, scarlet runner bean
Pisum sativum, pea (field or garden)
Vicia faba, horse bean or broadbean
Vicia spp., vetch
Vigna angularis, adzuki bean

a General description

Seed unit: True seed, dicotyledonous.

Germination habit: Hypogeal.

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

Shoot system: The epicotyl elongates and carries the terminal bud and primary leaves above the soil 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 soil; 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.

Root system: A long primary root with secondary roots.

b 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

-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

c Substrate effects

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

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

d Remarks

The percentage of hard seeds is to be reported in addition to the percentage germination. (Rules for Testing Seeds, 4.2.d)

If at the end of the germination period there are swollen seeds present, or seeds which have just started to germinate, remove everything but these seeds and continue the test. After five additional days, add any normal seedlings to the previous count (Rules for Testing Seeds, 4.9.d(6), 4.9.k(6)).

Mechanical damage is a common cause of abnormalities in field peas. Very small seedlings should be examined closely to determine if the small size is a result of breaks at the point of attachment of the cotyledons. Such seedlings are to be classified as abnormal.

Manganese deficiency at the time of seed development may cause a condition known as "marsh spot", characterized by a discolored 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 should be retested in soil.

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.

4.6.3 Fabaceae - Soybean and lupine

Glycine max, soybean
Lupinus spp., lupine

a General description

Seed unit: True seed, dicotyledonous.

Germination habit: Epigeal.

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 soil 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.

b 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 remarks)

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 or adventitious roots

Seedling:

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

-albino

c Substrate effects

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.

The epicotyl may show yellowish or brownish areas under certain test conditions (eg. excessive humidity), making evaluation difficult. When a number of seedlings are observed with this condition the sample should be retested.

Roots of seedlings on Kimpak under dry test conditions may not become established and hypocotyl elongation may appear to be abnormal. There may be curling of the root and hypocotyl. When a number of seedlings are observed with this condition, the sample should be retested.

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 may be advisable.

d Remarks

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 soil or sand, will aid in interpretation of such seedlings.

Hypocotyl development is slow until the roots start functioning; caution should 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 soil or sand, will aid in interpretation of such seedlings.

A five day preliminary count is permissible, but caution should be exercised to avoid the possibility of misinterpreting small seedlings or causing damage. It is also permissible to extend the test from 2 to 5 days (Rules section 4.9d(4 and 6)) where slow growth or substrate effects have occurred.

Dormancy seldom occurs but some seeds may be slower to start. Hard seeds may be present and are to be reported in addition to the percentage germination (Rules 4.2.d).

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.

4.6.4 Fabaceae - Peanut

<u>Arachis hypogaea</u>, peanut

a General description

Seed unit: True seed (may be received in the pod, which should be removed before testing). Dicotyledonous.

Germination habit: Epigeal.

Food reserves: Cotyledons, which are large and fleshy.

Shoot system: The cotyledons are carried to the soil surface by the hypocotyl which is very thick, narrowing abruptly just above the root. Elongation of the hypocotyl stops when the epicotyl is exposed to light at the soil surface; often elongation stops before the cotyledons have broken through the surface. The primary leaves are compound and usually expand during the test period, although the epicotyl may be dormant.

Root system: A long primary root with secondary roots. Adventitious roots develop from the base of the hypocotyl if the primary root is damaged.

b 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

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 or adventitious roots

Seedling:

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

-albino

c Substrate effects

Hypocotyls remain somewhat thickened and may appear to be stunted. Light, depth of planting and substrate moisture all contribute to the length of the hypocotyl.

Hypocotyl stunting and curling may be caused by constriction and/or seedling orientation on or in the substrate.

Seedlings planted in a soil test with the radicle too close to the surface may send roots above the soil and appear to exhibit negative geotropism and a distorted hypocotyl (U-shaped).

If necessary for the seedling to reorient itself for emergence, it may form a neck which includes the base of the cotyledons. In this case both cotyledons will be bent to one side and carried well above the soil surface. If oriented with the radicle pointing downward, the seedling may not form a neck but will push the cotyledons directly up to the surface.

d Remarks

Freshly harvested peanuts may exhibit varying degrees of dormancy. It is often necessary to resort to artificial means of breaking dormancy in order to achieve satisfactory germination (see Rules 4.9g and 4.9h)

4.6.5 Fabaceae - Small-seeded

<u>Alysicarpus</u> <u>vaginalis</u>, alyceclover
<u>Astragalus</u> <u>cicer</u>, cicer milkvetch
<u>Coronilla</u> <u>varia</u>, crownvetch
<u>Crotalaria</u> spp., crotalaria
<u>Cyamopsis</u> <u>tetragonoloba</u>, guar
<u>Desmodium</u> <u>tortuosum</u>, hairy indigo
<u>Hedysarum</u> <u>boreale</u>, northern sweetvetch
<u>Indigofera</u> <u>hirsuta</u>, hairy indigo
<u>Kummerovia</u>, lespedeza
<u>Lespedeza</u> spp., lespedeza
<u>Lotus</u> spp., trefoil
<u>Medicago</u> <u>arabica</u>, spotted burclover
<u>Medicago</u> <u>lupulina</u>, black medick
<u>Medicago</u> <u>orbicularis</u>, button clover
<u>Medicago</u> <u>polymorpha</u>, California bur-clover
<u>Medicago</u> <u>sativa</u>, alfalfa
<u>Melilotus</u> <u>indica</u>, sourclover
<u>Melilotus</u> spp., sweetclover
<u>Onobrychis</u> <u>viciifolia</u>, sainfoin
<u>Pueraria</u> <u>lobata</u>, kudzu
<u>Sesbania</u> <u>macrocarpa</u>, sesbania
<u>Trifolium</u> spp., clover

a General description

Seed unit: True seed, dicotyledonous; one and two-seeded pods; burs of the bur clovers.

Germination habit: Epigeal.

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

Shoot system: The hypocotyl elongates and carries the cotyledons above the soil 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.

b 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
-weak and watery

Root:

-none
-primary root stubby (for sweet clover, or for roots bound by the seed coat see comments under substrate effects)
-split extending into the hypocotyl

Seedling:

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

c Substrate effects

Stubby roots when germinated on artificial media:

a) 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 soil, such seedlings are to be classified as normal.
b) 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.

d Remarks

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 should be peeled back to determine whether a break has occurred.

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.

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.

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 should be allowed an additional five days as provided in the Rules (4.9d). For swollen seeds of alyce clover, see Rules section 4.8a. Swollen seeds are an indication of dormancy and can be induced by incorrect temperatures.

4.7 Liliaceae, Lily family

4.7.1 Liliaceae - Asparagus

Asparagus officinalis, asparagus

a General description

Seed unit: True seed, monocotyledonous

Germination habit: Hypogeal

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 soil 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.

b 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

Hypocotyl:
-not evaluated

Root:

-no primary root
-stubby primary root, with weak secondary roots (but see remarks for ornamental asparagus)

Seedling:

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

c Remarks

Several epicotyls may arise simultaneously and may be considered normal if at least one appears to be vigorous and has a terminal growing point.

Some seeds do not contain an embryo. Firm seeds may be cut and tested for viability (Rules 4.9.k) to determine the presence of a viable embryo. Viable ungerminated seeds may be reported as dormant.

The ornamental asparagus (Asparagus setaceus) has a thickened bulb-like primary root, in contrast to the long, slender root of the garden asparagus (A. officinalis).

4.7.2 Liliaceae - Onion, leek and chives

Allium cepa, onion
Allium porrum, leek
Allium schoenoprasum, chives

a General description

Seed unit: True seed, monocotyledonous.

Germination habit: Epigeal.

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 soil surface. The cotyledon tip is pulled from the soil 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.

b 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

c Substrate effects

Excess moisture may cause a delay in germination causing some seed lots to appear dormant.

d Remarks

Blotter or towel tests of onion are commonly overcome with fungus. To reduce this problem on a retest, seeds should be spaced farther apart.

4.8 Linaceae, Flax family

4.8.1 Linaceae - Flax

Linum usitatissimum, flax

a General description

Seed unit: True seed, dicotyledonous.

Germination habit: Epigeal.

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 soil 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.

b 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

4.9 Malvaceae, Mallow family

4.9.1 Malvaceae - Okra and cotton

Abelmoschus esculentus, okra
Gossypium spp., cotton

a General description

Seed unit: True seed, dicotyledonous.

Germination habit: Epigeal.

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 soil 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.

b 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

c **Remarks**

Seedlings with yellowish areas on the roots or hypocotyls of cotton are to be classified as normal, provided the cotyledons are free of infection. Seed coats must be removed from young seedlings to determine this.

Delinting of cotton by acid or flame may cause extensive damage to the cotyledons. The damaged cotyledons are very susceptible to decay and the seed coats may remain attached. It is essential that the cotyledons be freed from the seed coat so that the extent of damage can be evaluated.

4.10 Poaceae, Grass family

4.10.1 Poaceae - Cereals

Avena _sativa_ and _A._ _byzantina_, oat
Hordeum _vulgare_, barley
Secale _cereale_, rye
Secale _montanum_, mountain rye
Triticum spp., wheat
_X_ _Triticosecale_, triticale

a General description

Seed unit: Caryopsis, contained within the lemma and palea for some species (oat and barley).

Germination habit: Hypogeal

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 transfering them to the growing seedling.

Shoot system: The epicotyl consists of the coleoptile and enclosed leaves which grow from the meristematic region at their base. The epicotyl elongates and pushes through the soil surface; the mesocotyl may elongate depending on the variety and light intensity, but is usually not discernible.

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.

b Abnormal seedling description

Epicotyl:
- -missing
- -no leaf
- -leaf extending less than halfway up into the coleoptile
- -leaf badly shredded or longitudinally split
- -thin, spindly, pale or watery

Mesocotyl (if visible):
- deep open cracks

Root:
- less than one strong seminal root.

Seedling:
- decay at point of attachment to the scutellum
- one or more essential structures impaired as a result of decay from primary infection
- albino
- scutellum obviously detached from the endosperm (e.g. kernel lifted away by the growing shoot) and the seedling very weak in comparison with other seedlings in the same test.

c Substrate effects

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 epicotyl.

d Remarks

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 should be examined closely to determine if they have been damaged.

4.10.2 Poaceae - Rice

Oryza sativa, rice

a General description

Seed unit: Caryopsis, floret or spikelet.

Germination habit: Hypogeal.

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 epicotyl consists of the coleoptile and enclosed leaves which grow from the meristematic region at their base. The epicotyl elongates and pushes through the soil or water surface; the mesocotyl may elongate depending on the variety and environmental conditions.

Root system: Strong primary root and seminal roots. Adventitious roots may start to develop from the mesocotyl or coleoptilar node within the test period. If the mesocotyl elongates the adventitious roots will be carried above the grain. (Also see Remarks)

b Abnormal seedling description

Epicotyl:

-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)

Mesocotyl (if visible):

-deep open cracks

Root:

-none
-weak primary root

Seedling:

- -decay at point of attachment to the scutellum
- -one or more essential structures impaired as a result of decay from primary infection
- -albino

c Substrate effects

Fungal development may cause variation in test results; more uniform results will be obtained if seeds are well spaced or grown in sand or soil. The "flood test" may also be used: The seed is planted in moist sand. On the seventh day of test add water to a depth of one quarter inch above the sand level and leave for the remainder of the test period. Only a final count is made.

d Remarks

Preliminary counts should not be made until the 5th to 7th day, since the epicotyl is not sufficiently developed to evaluate earlier.

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 should be examined closely to determine if they have been damaged.

All rice planting in California and much of the southern United States is by direct seeding of imbibed seeds into water. The seed itself is on the soil surface and the tip of the developing shoot emerges from the water at about the 3- or 4-leaf stage. The mesocotyl does not elongate in submerged plantings. The adventitious roots become the principal root system in later stages of growth, but when seeding is directly into water the primary and seminal roots are essential to anchor the developing seedling. Root growth is slower in submerged seedlings than in drilled plantings. Without the primary root, seedlings drift, resulting in erratic development and stand establishment. The primary root may extend to 15 cm. in length and is functional through the 7-leaf stage.

4.10.3 Poaceae - Corn

Zea mays, corn

a General description

Seed unit: Caryopsis

Germination habit: Hypogeal

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 transfering them to the growing seedling.

Shoot system: The epicotyl consists of the coleoptile and enclosed leaves which grow from the meristematic region at their base. The epicotyl elongates and pushes through the soil surface. The mesocotyl usually elongates.

Root system: Strong primary root and seminal roots. Adventitious roots may start to develop from the mesocotyl or coleoptilar node within the test period.

b Abnormal seedling description

Epicotyl:
- -missing
- -no leaf
- -leaf extending less than halfway up into the coleoptile
- -leaf badly shredded or longitudinally split

Mesocotyl (if visible):
- -deep open cracks

Root:
- -none
- -weak, stubby or missing primary root with weak seminal roots

Seedling:
- -thin, spindly, pale or watery shoot (in comparison with other seedlings in the test)
- -decayed at point of attachment to the scutellum

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

c Substrate effects

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.

d Remarks

Splitting from the tip 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 should be examined closely to determine if they have been damaged.

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).

A twisted and curled epicotyl bound by a tough seed coat may be considered normal.

4.10.4 Poaceae - Sorghum

Sorghum cv 'sorgrass'
Sorghum bicolor vars., sorghum
Sorghum halepense, Johnsongrass
Sorghum X almum, almum sorghum
Sorghum X drummondii, sudangrass

a General description

Seed unit: Spikelet

Germination habit: Hypogeal

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 transfering them to the growing seedling.

Shoot system: The epicotyl consists of the coleoptile and enclosed leaves which grow from the meristematic region at their base. The epicotyl elongates and pushes through the soil surface; the mesocotyl usually elongates.

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.

b Abnormal seedling description

Epicotyl:
- -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)

Mesocotyl (if visible):
- -deep open cracks

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

c Remarks

Preliminary counts should not be made until the 4th or 5th day.

Seedlings with red coloration on or in the roots or coleoptiles caused by natural pigmentation are to be considered normal.

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 should be examined closely to determine if they have been damaged.

4.10.5 Poaceae - Other kinds

All grass species listed in the Rules, other than barley, corn, oats, rice, rye, Sorghum, triticale and wheat.

a General description

Seed unit: Caryopsis, floret, multiple floret or spikelet, depending on the species.

Germination habit: Hypogeal

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 transfering them to the growing seedling.

Shoot system: The epicotyl consists of the coleoptile and enclosed leaves which grow from the meristematic region at their base. The epicotyl elongates and pushes through the soil surface. The mesocotyl usually does not elongate significantly in most species, but may in some (e.g. tall oatgrass).

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.

b Abnormal seedling description

Epicotyl:

-missing
-short, thick and grainy
-no leaf
-leaf extending less than halfway up into the coleoptile
-leaf badly shredded or longitudinally split

Mesocotyl (if visible):

-deep open cracks

Root:

-no primary root
-spindly, stubby or watery primary root (for Kentucky Bluegrass, see remarks)

Seedling:

-thin, spindly, pale or watery shoot (in comparison with other seedlings in the test)
-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)
-scutellum obviously detached from the endosperm (e.g. kernel lifted away by the growing shoot) and the seedling very weak in comparison with other seedlings in the same test.

c Substrate effects

The use of a potassium nitrate solution is recommended for breaking dormancy. Its use may cause shortened roots and promote fungal growth. Retests on top of soil in closed petri dishes are recommended to aid in interpretation of short roots which appear to be caused by the use of potassium nitrate.

d Remarks

Seedlings are to be classified as abnormal if the primary root is defective even if other roots are present.

In certain species (eg. Bermudagrass) 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 should be removed and the root observed. Such seedlings are to be classified as normal if the root has developed.

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 should be examined closely to determine if they have been damaged.

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.

For Kentucky Bluegrass, seedlings with a primary root 1/16 inch or more in length are to be classified as normal.

4.11 Miscellaneous Agricultural and Horticultural

4.11.1 Miscellaneous - All kinds

Apiaceae, carrot family - carrot, celery, celeriac, parsley, parsnip
Cannabinaceae, hemp family - hemp
Dichondraceae, dichondra family - dichondra
Geraniaceae, geranium family - alfilaria
Pedaliaceae, benne family - sesame
Polygonaceae, knotweed family - buckwheat, sorrel, rhubarb
Rosaceae, rose family - little burnet
Solanaceae, nightshade family - eggplant, tomato, husk tomato, pepper, tobacco
Valerianaceae, valerian family - cornsalad

a General description

Seedlings are considered normal if they possess those essential structures that are indicative of its ability to produce a plant under favorable conditions.

b 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

-missing or stubby primary root with weak secondary or adventitious roots

4.12 Trees and Shrubs

4.12.1 Trees and shrubs - All kinds

a General description

Seedlings are considered normal if they possess those essential structures that are indicative of its ability to produce a plant under favorable conditions.

b Abnormal seedling description

- Weak, rootless or broken
- With poor or stunted radicle growth
- With radicle emerging from the seed coat with apparently normal development of hypocotyl and cotyledons, but radicle failing to develop.
- With radicle emerging from the seed coat but growing horizontally or upward.
- With radicle emerging from the seed coat but being destroyed by rapid growth of fungus from within the seed coat.
- With cotyledons emerging from the seed coat before the radicle.
- Seed split from internal growth with nothing emerging or with only short blunt extension of endosperm and radicle.
- With stunted hypocotyl carrying a "collar" of endosperm tissue.
- With double embryos fused together. (If two seedlings emerge from a seed and they are not fused, or one is normal or produces a completely normal plant, the unit would not be classed as abnormal).
- Without pigment - albino.
- Watery - translucent in appearance.

5 Glossary

Abnormal seedling.
A seedling that does not have all the essential structures or is damaged, deformed or decayed to such an extent that normal development is prevented (see normal seedling).

Achene.
A dry, hard, one-chambered, one-seeded indehiscent fruit, as in buckwheat, lettuce and spinach.

Adventitious root.
A root arising from any structure other than a root (e.g. a stem-borne root).

Albino.
A seedling in which all tissues are white due to the absence of pigments.

Caryopsis.
The single-seeded fruit or grain of the grass family (Poaceae); the fruit wall (pericarp) is united with the seed coat (testa).

Coleoptile.
The sheath enclosing the terminal bud of the embryo and the developing leaves of the young seedling of the grass family (Poaceae).

Coleorhiza.
The sheath enclosing the radicle of the grass embryo.

Conducting tissues.
Tissues which transport water and dissolved minerals from the root to the other plant structures, and foods from where they are manufactured (e.g. leaves) to where they are needed for growth or storage.

Cotyledon.
The non-foliar leaf or pair of leaves of an embryo and seedling (see primary leaf).

Decay.
Break-down of organic tissue, usually associated with the presence of micro-organisms.

Dicotyledons.
A group of plants so classified because the embryo usually has two cotyledons (see monocotyledons).

Diseased.
Showing symptoms of the presence and activity of pathological or detrimental micro-organisms.

Embryo.
A rudimentary plant contained in a seed, usually consisting of a more or less differentiated axis and attached cotyledon(s).

Endosperm.
Nutritive tissue originating from fertilization and retained at maturity in some seeds as storage for food reserves.

Epicotyl.
The part of the seedling above the cotyledons, consisting of the epicotyl stem, the developing leaves and the terminal bud.

Epigeal germination.
A type of germination in which cotyledons are carried above soil level by the elongating hypocotyl (see hypogeal germination).

Geotropism.
Plant growth response to gravity.

Hypocotyl.
The part of the seedling above the primary root and below the cotyledons.

Hypogeal.
A type of germination in which the cotyledon(s) or comparable structure (e.g. scutellum) remain in the soil (see epigeal germination).

Imbibition.
The uptake of water by the seed from the germination substrate.

Impaired.
Unable to function normally, in reference to damaged seedling structures.

Infection.
Entrance and spread of disease organisms in living material (e.g. seedling structures) often causing disease symptoms and decay.

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

The first leaf or leaves above the cotyledons.

Primary root.
Main root of the seedling, developing from the radicle of the embryo.

Radicle.
The rudimentary root of the embryo, developing into the primary root after emergence from the seed.

Root hair.
Fine tubular growth from an epidermal cell of a root.

Scale leaf.
A reduced leaf, usually appressed to the stem (e.g. in Asparagus, Pisum).

Scutellum.
A shield shaped cotyledonary structure in the embryo of the grasses (Poaceae) which serves to absorb nutrients from the endosperm.

Secondary infection.
See infection.

Secondary root.
Any root other than primary, seminal or adventitious roots.

Seed unit.
The structure usually regarded as a seed in planting practices and in commercial channels, consisting of a true seed with or without accessory structures, as defined in Section 2.6 of the Rules for Testing Seeds. See also true seed.

Seedling.
A young plant developing from the embryo of a seed.

Seminal roots.
Roots arising from the scutellar node in Poaceae.

Shoot.
A collective term including all structures above the root in epigeal species and above the cotyledonary node in hypogeal species.

Spindly.
Disproportionately thin relative to length; thread-like in appearance.

Stubby root.
Blunt, broken off or dwarfed.

Terminal bud.
The apical meristem of the epicotyl enveloped by several more or less differentiated leaves.

Testa.
Seed coat. The covering structure of a true seed; derived from the integument(s).

True seed.
A mature ovule consisting of an embryo, with or without an external food reserve (e.g. endosperm) enclosed by the testa.

Vascular tissues.
See conducting tissues.