AOSA Rules Changes Accepted
at the 1985 Annual Meeting
in Richmond, Virginia

by
Arnold L. Larsen
Chairman, Rules Committee

Seventeen Rule change proposals were presented to the membership of AOSA at Richmond, Virginia. Sixteen were accepted and one rejected. Listings of the old rules, changes proposed and justification for the proposals are found in AOSA Newsletters 59(1):17-39 and 59(2):19-23. Some moderate adjustments were made to a few of the proposals at the annual meeting, therefore, the accepted rule changes with adjustments are as follows:

1. Concerns the official nature of the "rules" and of documents referred to therein.

    Insert as last paragraph in the Introduction Section on page 1. This document constitutes the official AOSA statement regarding seed testing procedures and is referred to as the "rules". Changes in either the rules or to documents referred to in the rules cannot be considered official until the changes are accepted by the AOSA membership in the general business meeting at an annual convention of the association.

2. Concerns the working weight for noxious weed exams.

    2.3a. Same as present rule but add the following:
    When a purity analysis is performed on a sample, the weight of the sample used for purity analysis may be considered part of the minimum weight specified for the noxious weed seed examination.

3. Concerns "variant" and "off-type" seeds in Kentucky bluegrass seed lots.

    This proposal was rejected.

4. Concerns a deletion of a special exemption for Dallisgrass (_Paspalum dilatatum_) and Bahiagrass (_P. notatum_).

    2.7h. Seed units with nematode galls, fungus bodies (i.e., ergot, smut, etc.) and spongy or corky caryopses which are entirely enclosed within the seed unit. Refer to section 2.10a(8) and c(1).

    AND

    2.10a(8) Seed units with nematode galls or fungus bodies (smut, ergot, and other sclerotia) which are not entirely enclosed within the seed unit. Refer to section 2.7h.

5. Concerns adjustment in AOSA Handbook 24 needed for Pensacola bahiagrass (_Paspalum notatum_ cv.'Pensacola') to accomodate the change in Rules sections 2.7h and 2.10a(8).

    AOSA Handbook 24. The Uniform Blowing Procedure, 1983 revision.
    6.5 Procedure For Purity Analysis of Pensacola Bahiagrass(_Paspalum notatum_ cv. 'Pensacola')
    STEP 2. Separating the Heavy Fraction

Other crop seed (including bahiagrass cultivars other than 'Pensacola'), weed seed and seed like particles and inert matter (sticks, sand etc.) are classified in accordance with sections 2.7-2.10 of the Rules.

All Pensacola bahiagrass seed units, including multiple florets and free caryopses, shall be considered pure seed. Also, broken seeds which are less than half of their original size are inert.

6. Concerns source of calibration samples.

   2.11c. Calibration samples: Calibration samples for Kentucky bluegrass, orchardgrass, and Pensacola variety of bahiagrass are to be used to establish a blowing point prior to proceeding with the separation of pure seed and inert matter. Calibration samples for all three species with instructions, are available on a loan basis from the Federal Seed Laboratory, AMS,USDA, Bldg. 306, Rm. 213, Beltsville, MD 20705. Kentucky bluegrass and orchardgrass calibration samples may be purchased through the AOSA Secretary-Treasurer.

7. Concerns blower restriction for rough bluegrass.

   2.11 d(3) Rough bluegrass: The blower setting obtained for the Kentucky bluegrass calibration sample multiplied by a factor of 0.82 shall be used (The 0.82 factor is restricted to the General-type Seed Blower, see section 3.3(a) AOSA Handbook 24.)

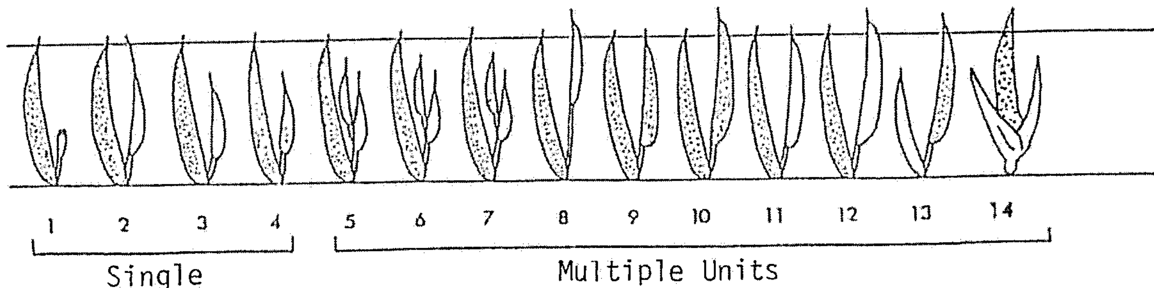
8. Concerns deletion of Handbook 24 reference in section 2.11d(5).

   2.11 d(5) Orchardgrass: The blower setting obtained by the Orchardgrass calibration sample shall be used.

9. Concerns further changes in multiple unit definition.

   2.12b Definition: A multiple unit is a seed unit that includes one or more structures as follows:
   (1) An attached sterile or fertile floret that extends to or beyond the tip of a fertile floret (structures 8-12);
   (2) A fertile floret with basally attached glume, glumes, or basally attached sterile floret of any length (structures 13-14);
   (3) A fertile floret with two or more attached sterile fertile florets of any length (structures 5-7).
   The length of an awn shall be disregarded when determining the length of a fertile floret or an attached structure. Any seed unit without attached structures, as described above, shall be considered a single unit (structures 1-4).

The stippled (dotted) portion represents fertile florets and the clear portion represents sterile florets or glumes.



10. Concerns a new method of distinguishing between yellow and white sweetclover which will replace the mottled seed count method.

3.4 Chemical test to distinguish sweetclover. In determining admixtures of yellow sweetclover and white sweetclover, at least 400 seeds shall be subjected to the chemical test as follows:

a. Preparation of test solution - Add 3 grams of cupric sulfate ($CuSO_4$) to 30 ml of household ammonia (NH_4OH, approx 4.8%) in a stoppered bottle to form the tetraamminecopper sulfate ($Cu(NH_3)_4SO_4$) solution used for this test. After mixing, a light blue precipitate of cupric hydroxide ($Cu(OH)_2$) should form. If no precipitate forms, add additional $CuSO_4$ until a precipitate appears. Since the strength of household ammonia can vary, this insures that a complete reaction takes place between $CuSO_4$ and NH_4OH; otherwise fumes from excess ammonium hydroxide may cause eye irritation.

b. Preparation of seeds - To insure imbibition, scratch, prick, or otherwise scarify the seed coats of the sweetclover seeds being tested. Imbibe seeds in water for 2 to 5 hours in a glass container.

c. Chemical reaction - When seeds have imbibed, remove excess water and add enough test solution to cover the seeds. Seed coats of yellow sweetclover will begin to stain dark brown to black; seed coats of white sweetclover will be olive or yellow-green. Make the separation within 20 minutes, since the seed coats of white sweetclover will eventually turn black also.

d. Calculation of results - Count the number of seeds which stain dark brown or black and divide by the total number of seeds tested; multiply by the pure seed percentage for *Melilotus* spp. the result is the percentage of yellow sweetclover in the sample. The percentage of white sweetclover is found by subtracting the percentage of yellow sweetclover from the percentage of *Melilotus* spp. pure seed.

Example:

Pure *Melilotus* spp. = 98.76%
Number of seeds tested = 400
Number of seeds staining dark brown or black = 32

% Yellow sweetclover = (32/400) X 98.76% = 7.90%
% White sweetclover = 98.76% - 7.90% = 90.86%

11. Concerns the expansion of the dormant seed definition to all species

4.2 Definitions

e. Dormant seed - Viable seeds, other than hard seeds, which fail to germinate when provided the specified germination conditions for the kind of seed in question. Viability of ungerminated seeds of all species listed in Tables 3, 4, and 5 may be determined by any appropriate method or combination of methods. The percentage dormant seeds, if present, may be reported in addition to the percentage germination. Refer to 4.9k. If the presence of dormant seeds is suspected but not determined the statement "viability of ungerminated seeds not determined" should be written on the germination analysis report.

12. Concerns families containing hard seed

4.9d (6) If at the end of the germination period provided for species belonging to Convolvulaceae, Geraniaceae, Malvaceae and Fabaceae, there are still swollen seeds present, or seeds which have just started to germinate, all seeds or seedlings except the above stated shall be removed and the test continued for five additional days. Any additional normal seedlings shall be included in determining the percentage of germination. Refer to section 4.9k(6).

13. Concerns a new section (4.9k) on determining viability of seed remaining ungerminated at the end of the prescribed germination test period.

4.9k Viability testing of ungerminated seed. - Any of the following methods or combination of methods, unless otherwise specified, may be used to determine the viability of ungerminated seed which remain at the end of the prescribed test period. The results are to be reported as percentage dormant or hard seed as determined by the specified method.

(1) Embryo excision test (EE) - Principles and procedures may be found in the following literature a.) "Reliability of the excised embryo method as a rapid test for determining the germinative capacity of dormant seed." F. Flemion, 1948. Boyce Thompson Institute for Plant Research Inc. 229-241. b.) "The excised embryo method for testing germination of dormant seed." C. E. Heit, 1955. Proc. Assoc. Seed Anal. 45:108-117. and c.) "Provisional rules for excised embryo test." Appendix C. 1976 Seed Sci. and Technol. 4(1) 174-177. The test may be placed at the prescribed temperature or at room temperature if maximum temperature does not exceed 24° C.

(2) Tetrazolium test (TZ) - Principles and procedures may be found in the following literature: a.) "Tetrazolium testing handbook for agricultural seeds" D. F. Grabe, ed. AOSA Handbook No. 29, 1970 62p and b.) "Biochemical test for viability." Annexe to Chapter 6. Seed Sci. and Technol. 4(1) 133-159.

(3) Scarification - For tree and shrub species listed in Table 5 impermeable seed coats of ungerminated seeds may be modified by either mechanical or chemical scarification. The seed may be clipped, filed or pierced opposite the radicle end, or rubbed with an abrasive material, i.e. sandpaper.

Dry seed may be placed in concentrated sulfuric acid (H_2SO_4) for the prescribed length of time, rinsed thoroughly in running tap water and then returned to the test condition.

Caution: Concentrated sulfuric acid is caustic and can cause severe skin burns and produce holes in clothing on contact. It is advisable to wear rubber gloves and protective clothing while working with this chemical.
Note: When rinsing acid treated seed always add acid to water. Heat produced by the chemical reaction of adding water to the acid may injure or kill the seed.

(4) Germination promoting chemicals: Gibberellic Acid (GA_3) - (ISTA formulation - Seed Sci. and Technol., 4(1) pg. 112.) The germination substratum should be moistened with the recommended concentration, 200 PPM-500 PPM of GA_3 for most cases. Stronger solutions may be used for stronger cases of dormancy. When the concentration is higher than 800 PPM the use of a buffer is recommended. A 500 PPM solution of GA_3 is prepared by dissolving 500 mg. GA_3 in one liter of water.

(5) Cutting test for tree and shrub seeds (Table 5) - The seed is cut open and internal structures are observed. Fully developed, firm tissue with the proper coloring is considered viable while shriveled, decayed and discolored tissue or seed lacking an embryo is considered nonviable. If the seed has not been prechilled and an extremely high percent of viable seed is found, a retest of prechilled seeds may be advisable.

(6) Hard Seeds - The percentage of hard seed is to be reported in addition to the percentage germination. If swollen seeds or seeds which have started to germinate are present at the end of the prescribed germination period, remove all hard seeds (record their number) and for flat pea (Lathyrus sylvestris), continue the swollen seed in test for 14 days, when germinating at 15-25C or 10 days when germinating at 20C. For all other species listed in Tables 3, 4, and 5 continue the test for 5 additional days. The additional normal seedlings shall be included in the percentage of germination. Refer to section 4.9d(6).

For hard seeds in tree and shrub seed (Table 5) see 4.9k (3).

Other changes

4.6b. (Delete reference"..., such as the presence of firm ungerminated seeds, ...".
4.9d. (last paragraph) delete in this position and transfer concept to 4.9k (5).

Table 3 footnotes (page 58)
a. Replace "see sections 4.2e and 4.9" with "see sections 4.2d and 4.9k (6)".
d. Replace "see section 4.2e" with "see sections 4.2e and 4.9k".
e. Replace text following "Hard seeds often present" with "see section 4.9k (6)".

Table 4 footnotes (page 74)
b. Final count may vary with certain types, cultivars, or strains within any flower seed kind. Remaining seeds at the end of test should be critically examined for any viable seeds and recorded as dormant seeds (see 4.9k).
c. Replace text following "Hard seeds often present" with "see section 4.9k (6)".
d. Replace text with "Embryo excision method: see 4.9k (1)".

Table 5 footnotes (page 84)

a. Replace text with "Embryo excision method: see 4.9k (1)".
b. Replace text with "TZ tetrazolium: see 4.9k (2)".
c. Replace text with "Hard seed often present: see 4.9k (6)".

14. Concerns correcting inconsistancy in soybean germination procedure in Table 3.

In Table 3 section Vegetable and Herb seeds, add TC to the substrata list for Glycine max to conform to the list for the same species in the Agricultural seeds section.

15. Concerns references to photographs of seedlings.

4.9j footnote a: Only the photographs described in the AOSA Newsletter 57(3):67-72 (September 1983) may be purchased from the Office of Information, United States Department of Agriculture, Washington, D.C. 20250.

16. Concerns changes in seedling descriptions for lettuce (*Lactuca sativa*)

Appendix 1, Seedling descriptions, for lettuce was amended as follows:
2. Asteraceae, sunflower family
A. *Lactuca sativa*, lettuce

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.

Colored photographs and an interpretive drawing are available from the Federal Seed Laboratory, Beltsville, Maryland, and should be used as guides to classification of lettuce seedlings. Magnification up to 7X may be used for interpretations. Remove attached seed coats for seedling evaluation.

The following interpretations are to be made only at the end of the test period:

Normal seedling

Root	Strong primary root, usually with root hairs and with no splits or lesions.
Hypocotyl	Strong, with no cracks or lesions extending into the conductive tissues.
Cotyledons	(a) Two (b) If necrosis or injury is present, classify as normal if the necrosis or injury covers less than half the total cotyledon area.
Epicotyl	Present and entirely free from necrosis or decay. (May be assumed to be present if cotyledons are normal.)

Abnormal seedling

Root	(a) Primary root missing, damaged or weak. (b) Tips blunt, swollen, discolored. (c) Splits or lesions.
Hypocotyl	Severely twisted, grainy or with cracks or lesions extending into the conductive tissue.
Cotyledons	(a) Only one (b) Necrosis or injury covering one half or more of the total cotyledon area. (c) Swollen cotyledons usually associated with extremely short or vestigial hypocotyl and root.
Epicotyl	Missing or with any degree of decay.

17. Concerns testing for fungal endophyte (Acremonium spp.) in Tall fescue and other grass species.

9. FUNGAL ENDOPHYTE TESTING

9.1 Method of preparation of aniline blue stain for use in testing grass seed and plant material for the presence of Acremonium spp.

a. Prepare a 1% w/v aqueous aniline blue solution in water. (dilute 1 gram aniline blue to 100 ml. water).
b. Prepare a solution of one part of 1% aniline blue solution with 2 parts of lactic acid (85%).
c. Use stain as-is or dilute with water if sections are too dark.

9.2 Procedure for determining levels of Acremonium spp. in grass seed.

a. Take a sub-sample of seed (1 gram is sufficient)
b. Digest seed at room temperature for 12-16 hours in a 5% sodium hydroxide solution or other temperature/time combination resulting in adequate seed softening.
c. Rinse thoroughly in running tap water.
d. De-glume seed and place on microscope slide in a drop of seed stain. Slightly crush seed. Use caution to prevent carryover hyphae of Acremonium from one seed to another.
e. Place coverglass on seed and squash with gentle pressure.
f. Examine with compound microscope at 100-400x magnification, scoring a seed as positive if any identifiable hyphae are present.
g. Various sample sizes may be used for this test. Precision changes with sample size, therefore, the test results must include the sample size tested.
h. Test tolerances - see section 5.6, Table 12.

9.3 Procedure for determining levels of fungal endophyte (Acremonium spp.) in grass plant material.

a. Tillers from field stands.
   (1) Tillers must be randomly collected; one tiller each from each clump.
   (2) Samples should be free of contaminating fungi and other grasses such as annual ryegrass, orchardgrass and crabgrass.
   (3) Freezing will preserve samples and make subsequent peeling of tissue easier.
b. Seedlings from seeds suspected to contain fungal endophyte.
   (1) Select seeds at random and germinate.
   (2) Examine seedlings from the sample germinated after growing for a minimum of 48 days
c. Remove the outermost sheath from the tiller or seedling. Tissue should have no obvious discoloration from saprophytes and should have as little chlorphyll as possible.
d. Isolate a longitudinal section of sheath approximately 3-5mm in width.
e. Place the section on a microscope slide with the epidermis side down.
f. Stain immediately with aniline blue-lactic acid stain. Allow dye to remain at least 15 seconds but no more than one minute.

g. Blot off excess dye with tissue paper. Sections should remain on the slide, but may adhere to the tissue paper (If so, remove and place on proper position on the slide).
h. Place a coverglass on the sections and flood with water.
i. Examine section at 200x magnification. Score a section as positive if any identifiable hyphae are present.
j. Various sample sizes may be used for this test. Precision changes with sample size, therefore the test results must include the sample size tested.
k. Test tolerances - see section 5.6, Table 12.

5.6 Tolerance for endophyte testing

Table 12. Tolerances for fungal endophyte tests when results are based on 30 to 400 seeds, seedlings, or plants in a test.

Seed, seedling, or plant count percent	Number of seeds, seedlings, or plants in tests						
	30	50	75	100	150	200	400
100 or 0	0	0	0	0	0	0	0
98 or 2	6.0	4.6	3.8	3.3	2.7	2.3	1.6
96 or 4	8.3	6.4	5.3	4.6	3.7	3.2	2.3
94 or 6	10.1	7.8	6.4	5.5	4.5	3.9	2.9
92 or 8	11.5	8.9	7.3	6.3	5.2	4.5	3.4
90 or 10	12.8	9.9	8.1	7.0	5.7	4.9	3.8
88 or 12	13.8	10.7	8.7	7.6	6.2	5.4	4.1
86 or 14	14.7	11.4	9.3	8.1	6.6	5.7	4.5
84 or 16	15.5	12.1	9.8	8.5	7.0	6.0	4.8
82 or 18	16.4	12.6	10.3	8.9	7.3	6.3	5.0
80 or 20	16.9	13.2	10.7	9.3	7.6	6.6	5.3
78 or 22	17.6	13.6	11.0	9.6	7.9	6.8	5.5
76 or 24	18.2	14.1	11.5	9.9	8.1	7.0	5.7
74 or 26	18.6	14.4	11.8	10.2	8.3	7.2	5.8
72 or 28	19.0	14.8	12.1	10.5	8.5	7.4	6.0
70 or 30	19.5	15.1	12.3	10.7	8.7	7.5	6.2
68 or 32	19.9	15.4	12.5	10.8	8.9	7.7	6.3
66 or 34	20.2	15.7	12.7	11.0	9.0	7.8	6.4
64 or 36	20.5	15.8	12.9	11.2	9.1	7.9	6.5
62 or 38	20.6	15.9	13.0	11.3	9.2	8.0	6.6
60 or 40	20.9	16.1	13.2	11.4	9.3	8.1	6.7
58 or 42	21.0	16.2	13.3	11.5	9.4	8.1	6.8
56 or 44	21.0	16.4	13.3	11.5	9.4	8.2	6.8
54 or 46	21.2	16.4	13.4	11.6	9.5	8.2	6.9
52 or 48	21.2	16.5	13.4	11.6	9.5	8.2	6.9
50	21.3	16.5	13.4	11.6	9.5	8.2	6.9

AOSA Rules Committee (1984-85):
1985 Arnold L. Larsen (Chairman)
1986 Ellen M. Chirco
1987 Stan Kirkland
1988 Allen Knapp
1989 Stephen J. Hurst

Ex-officio members:
Robert W. Yaklich (Editorial)
Wayne R. Guerke (Research)
Mark W. Johnson (SCST)
Ellen M. Chirco (Referee)

17 SUBJECT:

Testing for fungal endophyte (Acremonium spp.) in Tall fescue and other grass species.

PRESENT RULE

None

PROPOSED RULE

9. FUNGAL ENDOPHYTE TESTING

9.1 Method of preparation of aniline blue stain for use in testing grass seed and plant material for the presence of Acremonium spp.

a. Prepare a 1% w/v aqueous aniline blue solution in water. (dilute 1 gram aniline blue to 100 ml. water).
b. Prepare a solution of one part of 1% aniline blue solution with 2 parts of lactic acid (85%).
c. Use stain as-is or dilute with water if sections are too dark.

9.2 Procedure for determining levels of Acremonium spp. in grass seed.

a. Take a sub-sample of seed (1 gram is sufficient)
b. Digest seed at room temperature for 12-16 hours in a 5% sodium hydroxide solution or other temperature/time combination resulting in adequate seed softening.
c. Rinse thoroughly in running tap water.
d. De-glume seed and place on microscope slide in a drop of seed stain. Slightly crush seed. Use caution to prevent carryover hyphae of Acremonium from one seed to another.
e. Place coverglass on seed and squash with gentle pressure.
f. Examine with compound microscope at 100-400x magnification, scoring a seed as positive if any identifiable hyphae are present.
g. Various sample sizes may be used for this test. Precision changes with sample size therefore the test results must include the sample size and also indicate the variability associated with that sample size. For this reason the following statement is required for reporting test results. "In this test, (number) seeds were examined and ___% were found to contain fungal endophyte hyphae. If subsequent tests are made with the same number of seeds, the results could vary between ___% and ___% at the 95% confidence level."
h. Test tolerances - see section 5.6, table 12.

9.3 Procedure for determining levels of fungal endophyte (Acremonium spp.) in grass plant material

a. Tillers from field stands.
   (1) Tillers must be randomly collect; one tiller each from each clump.
   (2) Samples should be free of contaminating fungi and other grasses such as annual ryegrass, orchardgrass, and crabgrass.
   (3) Freezing will preserve samples and make subsequent peeling of tissue easier.
b. Seedlings from seeds suspected to contain fungal endophyte.
   (1) Select seeds at random and germinate.
   (2) Examine all seedlings from the sample germinated.
c. Remove the outermost sheath from the tiller or seedling. Tissue should have no obvious discoloration from saprophytes and should have as little chlorophyll as possible.
d. Isolate a longitudinal section of sheath approximately 3-5mm in width.

e. Place the section on a microscope slide with the epidermis side down.
f. Stain immediately with aniline blue-lactic acid stain. Allow dye to remain at least 15 seconds but no more than one minute.
g. Blot off excess dye with tissue. Sections should remain on the slide, but may adhere to the tissue (If so, remove and place in proper position on the slide).
h. Place a coverglass on the sections and flood with water.
i. Examine section at 200x magnification. Score a section as positive if any identifiable hyphae are present.
j. Various sample sizes may be used for this test. Precision changes with sample size therefore the test results must include the sample size and also indicate the variability that is associated with that sample size. For this reason the following statements are required for reporting test results:
   (1) Report test results for tiller examination as follows: "In this test, (number) tillers were examined and ___% were found to contain fungal endophyte hyphae. If subsequent tests are made with the same number of tillers, the results could vary between ___% and ___% at the 95% confidence level.
   (2) Report test results on seedlings from planted seeds as follows: "In this test, (number) seeds were planted and ___% produced seedlings containing fungal endophyte hyphae. If subsequent tests are made with the same number of seeds, the results could vary between ___% and ___% at the 95% confidence level."

l. Test tolerances - see section 5.6, table 12.

5.6 Tolerance for endophyte testing

Table 12. Tolerances for fungal endophyte tests when results are based on 30 to 400 seeds, seedlings, or plants in a test.

Seed, seedling, or plant count percent	Number of seeds, seedlings, or plants in tests						
	30	50	75	100	150	200	400
100 or 0	0	0	0	0	0	0	0
98 or 2	6.0	4.6	3.8	3.3	2.7	2.3	1.6
96 or 4	8.3	6.4	5.3	4.6	3.7	3.2	2.3
94 or 6	10.1	7.8	6.4	5.5	4.5	3.9	2.9
92 or 8	11.5	8.9	7.3	6.3	5.2	4.5	3.4
90 or 10	12.8	9.9	8.1	7.0	5.7	4.9	3.8
88 or 12	13.8	10.7	8.7	7.6	6.2	5.4	4.1
86 or 14	14.7	11.4	9.3	8.1	6.6	5.7	4.5
84 or 16	15.5	12.1	9.8	8.5	7.0	6.0	4.8
82 or 18	16.4	12.6	10.3	8.9	7.3	6.3	5.0
80 or 20	16.9	13.2	10.7	9.3	7.6	6.6	5.3
78 or 22	17.6	13.6	11.0	9.6	7.9	6.8	5.5
76 or 24	18.2	14.1	11.5	9.9	8.1	7.0	5.7
74 or 26	18.6	14.4	11.8	10.2	8.3	7.2	5.8
72 or 28	19.0	14.8	12.1	10.5	8.5	7.4	6.0
70 or 30	19.5	15.1	12.3	10.7	8.7	7.5	6.2
68 or 32	19.9	15.4	12.5	10.8	8.9	7.7	6.3
66 or 34	20.2	15.7	12.7	11.0	9.0	7.8	6.4
64 or 36	20.5	15.8	12.9	11.2	9.1	7.9	6.5
62 or 38	20.6	15.9	13.0	11.3	9.2	8.0	6.6
60 or 40	20.9	16.1	13.2	11.4	9.3	8.1	6.7
58 or 42	21.0	16.2	13.3	11.5	9.4	8.1	6.8
56 or 44	21.0	16.4	13.3	11.5	9.4	8.2	6.8
54 or 46	21.2	16.4	13.4	11.6	9.5	8.2	6.9
52 or 48	21.2	16.5	13.4	11.6	9.5	8.2	6.9
50	21.3	16.5	13.4	11.6	9.5	8.2	6.9

REASONS FOR PROPOSAL:

1. Two states have seed laws that require testing and labeling as to the percent of fungal endophyte.
2. There are two states offering Tall fescue certification programs which certify for freedom from fungal endophyte.
3. There are a numberof new varieties of Tall fescue that are claimed to be free of fungal endophytes. Some new varieties require freedom from fungal endophyte to be sold by variety name.
4. There are state and commercial laboratories offering fungal endophyte testing for a fee. There are two large fungal endophyte testing laboratories associated with universities, each lab testing several hundred samples a year.
5. Workshops have been completed or are scheduled for Missouri, Alabama, Mississippi and Maryland. The method proposed here is the one used in all the workshops.
6. This method or similar methods are being used by animal diagnostic laboratories and by research personnel in most southern agricultural colleges to determine percent fungi.
7. Although all fungal endophyte testing procedures described in the scientific literature are not exactly the same, it is generally conceeded that the procedure proposed here is acceptable. Therefore the AOSA can appropriately assume the responsibility of standardizing endophyte testing procedures because this testing will undoubtedly be done in seed laboratories. Therefore this procedure should be placed in the AOSA Rules for Testing Seeds.
8. Since livestock losses are great and seed laws concerning fungal endophyte are being passed, there is some urgency that AOSA take a leadership position so that there is not a proliferation of testing procedures. AOSA also has the logistic capability of updating the testing procedures as better procedures are developed because they meet annually and therefore make adjustments annually.
9. The various analysts and scientists working with fungal endophyte testing can agree on general testing procedures but are not of accord on sample size. Therefore a range of sample sizes are listed in the proposed table 12 of section 5.6. It may be that plant material examinations will involve smaller sample sizes than seed examinations. However, precision changes with sample sizes,therefore,the sample size must be included with the test results as well as indication of the variability involved with that particular sample size. Hence the AOSA Rules Committee is imposing a report statement that includes the percent test results, sample size and extent to which a second test of the same size could vary.
10. The proposed section 5.6, Table 12 was taken from the Federal Seed Act, (Seed Testing Regulations), Section 201.62, Table 4 on page 46. Sample numbers 10,20, 800 and 1000 have been removed as being either unacceptably small or large.
11. The testing procedures in 9.3 for plant material are included for two reasons. First - a farmer may need to know if his Tall fescue field is contaminated with the fungal endophyte. Hence the report statement 9.3 j (1) involving tillers is proposed. Second - The test for fungal endophyte in seeds does not determine if the seeds or the fungal endophyte are viable. By testing for fungal endophyte in seedlings

from a certain seed sample size, the extent of living hyphae in living seeds can be determined. Hence the report statement in 9.3j(2) is proposed.

12. These proposed procedures may also be used to test ryegrass seed which is claimed to have fungal endophyte as a means of protecting the plant from insect attack.

SUBMITTED BY:

Fungal Endophyte Committee, Charles L. Sciple, Chairman

DATE:

January 25, 1985

Attachment 4

Multiple Unit Definitions

Original rule:

2.12 Definition: A multiple unit is defined as any unit possessing at least one fertile floret to which is attached any type of spikelet structure other than the rachilla.

Revised (1976):

2.12b Definition: A multiple unit is defined as any seed unit possessing a structure other than the rachilla which extends to or beyond the tip of the fertile floret. Any seed unit in which an attached structure is shorter than the fertile floret, shall be considered a single floret. The awn shall be disregarded when determining length of the fertile floret and attached structure.

Revised (1977):

2.12 Definition: A multiple unit is a seed unit that includes at least one fertile floret plus one or more of attached structure as follows:

(1) A sterile floret that extends to or beyond the tip of the fertile floret.

(2) Basally attached glume, glumes, or basally attached sterile florets of any length.

The length of an awn. . . . . etc.

Present (effective Oct. 1, 1984)

2.12b Definition: A multiple unit is a seed unit that includes <u>a</u> fertile floret plus one or more of attached structures as follows:

(1) no change

(2) no change

(3) one or more additionl fertile florets

The length of an awn. . . . . etc.

Proposed (FSL)

2.12b Definition: A multiple unit is a seed unit that includes one or more structures as follows:

(1) An attached sterile or fertile floret that extends to or beyond the tip of a fertile floret;

(2) A fertile floret with basally attached glume, glumes, or basally attached sterile floret of any length;

(3) A fertile floret with two or more attached sterile or fertile florets of any length.

The length of an awn. . . . . etc.

10. KIND OF SEED
Yellow sweetclover - *Melilotus officinalis*
White sweetclover - *Melilotus alba*

PRESENT RULE

3.4 Mottled seed in sweetclover - In determining admixtures of yellow sweetclover in white sweetclover, the percentage of mottled seed, by weight, shall be multiplied by four, and this product shall, in turn, be multiplied by the percentage of pure sweetclover in the sample. The product shall be construed as representing the percentage of yellow sweetclover. At least 400 seeds shall be used in making the determination.

PROPOSED RULE

3.4 Chemical test to distinguish sweetclover. In determining admixtures of yellow sweetclover and white sweetclover, at least 400 seeds shall be subjected to the chemical test as follows:

a. Preparation of test solution - Add 3 grams of cupric sulfate ($CuSO_4$) to 30 ml of household ammonia (NH_4OH, approx 4.8%) in a stoppered bottle to form the tetraamine copper sulfate ($[Cu(NH_3)_4]SO_4$) solution used for this test. After mixing, a light blue precipitate of cupric hydroxide ($Cu(OH)_2$) should form. If no precipitate forms, add additional $CuSO_4$ until a precipitate appears. Since the strength of household ammonia can vary, this insures that a complete reaction takes place between $CuSO_4$ and NH_4OH; otherwise fumes from excess ammonium hydroxide may cause eye irritation.

b. Preparation of seeds - To insure imbibition, scratch, prick, or otherwise scarify the seed coats of the sweetclover seeds being tested. Imbibe seeds in water for 2 to 5 hours in a glass container.

c. Chemical reaction - When seeds have imbibed, remove excess water and add enough test solution to cover the seeds. Seed coats of yellow sweetclover will begin to stain dark brown to black; seed coats of white sweetclover will be olive or yellow-green. Make the separation within 20 minutes, since the seed coats of white sweetclover will eventually turn black also.

d. Calculation of results - Count the number of seeds which stain dark brown or black and divide by the total number of seeds tested; multiply by the pure seed percentage for *Melilotus* spp; the result is the percentage of yellow sweetclover in the sample. The percentage of white sweetclover is found by subtracting the percentage of yellow sweetclover from the percentage of *Melilotus* spp. pure seed.

Example:
Pure *Melilotus* spp. = 98.76%
Number of seeds tested = 400
Number of seeds staining dark brown or black = 32

% yellow sweetclover = (32/400) X 98.76% = 7.90%
% white sweetclover = 98.76% - 7.90% = 90.86%

SUPPORTING EVIDENCE OR REASONS FOR THE PROPOSED CHANGE

Referees conducted by the Federal Seed Laboratory in 1981 and 1982 compared the mottled seed examination and a chemical method to distinguish seeds of yellow sweetclover and white sweetclover. The chemical test gave much more uniform results among laboratories than the mottled seed examination. Comparison with grow-outs showed that the chemical test gave more accurate results than the mottled seed examination.

There are several problems with the mottled seed examination. The intensity of the mottling varies; some mottling is so faint that magnification is required to see it. Although mottled seed is assumed to be yellow sweetclover, varieties of white sweetclover with mottling also exist. Another problem is the factor of four (4) which is part of the calculation of the percentage of yellow sweetclover. This was based on a study which found the average percentage of mottled seed in yellow sweetclover to be about 25%. Data from several subsequent studies showed that the percentage of mottled seed in yellow sweetclover can range from 2% to 72%. Therefore the validity of the factor of four is highly questionable.

Reference: Maxon, S.R. and S.J. Hurst. 1983. A comparison of methods to distinguish seeds of yellow sweetclover (*Melilotus officinalis* (*L.*) Lam.) and white sweetclover (*M. alba* Medik.). AOSA Newsletter 57 (1):46-53

SUBMITTED BY

Susan R. Maxon, Botanist, Federal Seed Laboratory, Building 306, Room 213, BARC-East, Beltsville, MD 20705

DATE: November 23, 1984.

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11. PROPOSAL

Dormant Seed Definition

PRESENT RULE

4.2 Definitions

e. Dormant seed - Viable seeds, other than hard seeds, which fail to germinate when provided the specified germination conditions for the kind of seed in question. Viability of ungerminated seeds may be determined by any appropriate method or combination of methods, such as a cutting test, tetrazolium test, scarification, and application of germination promoting chemicals. The percentage of dormant seeds, if present shall be reported in addition to the percentage germination for the following species: asparagus (*Asparagus spp.*), bahiagrass (*Paspalum notatum*), bluestems (*Andropogon gerardi*, *A. hallii*, *Bothriochloa ischaemum*, *Schizachyrium scoparium*), buffalograss (*Buchloe dactyloides*), buffelgrass (*Cenchrus ciliaris*), gramas (*Bouteloua spp.*) green needlegrass (*Stipa viridula*), Indian ricegrass (*Oryzopsis hymenoides*), lovegrasses (*Eragrostis spp.*), sand dropseed (*Sporobolus cryptandrus*), smilograss (*Oryzopsis miliacea*), switchgrass (*Panicum virgatum*), veldtgrass (*Ehrharta calycina*), western wheatgrass (*Agropyron smithii*), johnsongrass (*Sorghum halepense*), and indiangrass (*Sorghastrum nutans*).

PROPOSED RULE

4.2 Definitions

e. Dormant seed - Viable seeds, other than hard seeds, which fail to germinate when provided the specified germination conditions for the kind of seed in question. Viability of ungerminated seeds of all species listed in Tables 3,4, and 5 may be determined by any appropriate method or combination of methods. The percentage dormant seeds, if present, may be reported in addition to the percentage germination. Refer to 4.9k. If the presence of dormant seeds is suspected but not determined the statement "viability of ungerminated seeds not determined" should be written on the germination analysis report.

JUSTIFICATION

The list of species involved in this section is rapidly becoming unmanageable. There is a potential of more grasses, forbes, shrubs, among the range and revegetation species being included in the Rules. There are flowers, herbs and tree species seeds which are already listed in the rules as having dormant seeds but are not included in the list. A survey made by the AOSA Dormant Grass Seed Subcommittee revealed that dormant seed can be found in most of the species commonly tested by seed laboratories (AOSA Newsletter 44(3):50-57).

The wording of the proposal is written so that the determination of dormant seeds is optional. However, to protect both analysts and seedsmen whenever dormancy is suspected and not determined a statement should be made that dormancy was not investigated. This proposal also provides a means of determining total viability without deviating from the procedures listed in the rules. The use of unlisted procedures may mask dormancy and result in poor stands and increased variation in analysis of the same seeds among seed laboratories.

SUBMITTED BY

This proposal was prepared by Arnold L. Larsen in behalf of the AOSA Rangegrass Subcommittee and Ellen Chirco in behalf of the AOSA Flower Seed Committee.

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12. PROPOSAL

Families containing hard seed

PRESENT RULE

4.9 and paragraph following (5): if at the end of the germination period provided for legumes, cotton, okra and catnip, there are still swollen seeds present, or seeds which have just started to germinate, all seeds or seedlings except the above stated shall be removed and the test continued for five additional days. Any additional normal seedlings shall be included in determing the percentage of germination.

PROPOSED RULE

4.9 d (6) If at the end of the germination period provided for species belonging to Convolvulaceae, Geraniaceae, Malvaceae and Fabaceae, there are still swollen seeds present, or seeds which have just started to germinate, all seeds or seedlings except the above stated shall be removed and the test continued for five additional days. Any additional normal seedlings shall be included in determining the percentage of germination.

JUSTIFICATION

The hard seeded condition is associated with more species than indicated in the present rule therefore reference to intire familes appears to be in order. Convolvulaceae and Geraniaceae were added to the list because they are already footnoted as having hard seed in Table 4 (flower seeds). Catnip was excluded because it is controversial whether or not the mints are truly hard-seeded and also there is no record of having catnip included by a formal AOSA membership vote.

SUBMITTED BY

The proposal was prepared by Ellen Chirco of New York

DATE: July 24, 1984

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13. PROPOSAL

New section (4.9k) on determing viability of ungerminated seed.

PRESENT RULES

Sections regarding the methods for determining viability of ungerminated seeds after germination time has expired are distributed in different places:

Sections

4.2d Hard seed definitions
4.2e Dormant seed definitions
4.6b Retest if firm ungerminated seeds are present
4.8 Special procedures
4.9d (Second to last paragraph) hard & swollen seeds
4.9d (Last paragraph) tree and shrub seeds

Table 3 footnotes
a. Hard seed
d. Determine viability of ungerminated seed
e. Hard seed

Table 4 footnotes
b. Fresh firm, viable seed
c. Hard seed
d. Embryo excision

Table 5 footnotes
a. Embryo excision
b. TZ Tetrazolium
c. Hard seeds

PROPOSED RULE

Bring all statements regarding the methods of determining viability in remaining ungerminated seeds under one new heading. References throughout the rules for determining viability of ungerminated seeds will be made directly to this section as follows:

4.9k Viability testing of ungerminated seed. - Any of the following methods or combination of methods may be used to determine the viability of ungerminated seed which remain at the end of the prescribed test period. The results are to be reported as percentage dormant or hard seed as determined by the specified method.

(1) Embryo excision test (EE)- Principles and procedures may be found in the following literature a.) "Reliability of the excised embryo method as a rapid test for determining the germinative capacity of dormant seed." F. Flemion, 1948. Boyce Thompson Institute for Plant Research Inc. 229-241. b.) The excised embryo method for testing germination of dormant seed." C. E. Heit, 1955. Proc. Assoc. Seed Anal. 45:108-117. and c.) "Provisional rules for excised embryo test." Appendix C. 1976 Seed Sci. and Technol. 4(1) 174-177. The test may be placed at the prescribed temperature or at room temperature if maximum temperature does not exceed 24° C.

(2) Tetrazolium test (TZ) - Principles and procedures may be found in the following literature: a.) "Tetrazolium Testing handbook for agricultural seeds" D. F. Grabe, ed. AOSA Handbook No. 29, 1970. 62p and b.) "Biochemical test for viability." Annexe to Chapter 6. Seed Sci. and Technol. 4(1) 133-159. (Add ISTA Handbook if out of print.)

(3) Scarification - For tree and shrub species listed in Table 5 impermeable seed coats of ungerminated seeds may be modified by either mechanical or chemical scarification. The seed may be clipped, filed or pierced opposite the radicle end, or rubbed with an abrasive material, i.e. sandpaper.

Dry seed may be placed in concentrated sulfuric acid (H_2SO_4) for the prescribed length of time, rinsed thoroughly in running tap water and then returned to the test condition.

Caution: Concentrated sulfuric acid is caustic and can cause severe skin burns and produce holes in clothing on contact. It is advisable to wear rubber gloves and protective clothing ing while working with this chemical.
Note: When rinsing acid treated seed always add acid to water, Heat produced by the chemical reaction of adding water to the acid may injure or kill the seed.

(4) Germination promoting chemicals: Gibberellic Acid (GA_3) - (ISTA formulation - Seed Sci. and Technol., 4(1) pg 112.) The germination substratum should be moistened with the recommended concentration, 200 PPM - 500 PPM of GA_3 for most cases. Stronger solutions may be used for stronger cases of dormancy. When the concentration is higher than 800 PPM the use of a buffer is recommended. A 500 PPM solution of GA_3 is prepared by dissolving 500 mg. GA_3 in one liter of water.

(5) Cutting test for tree and shrub seeds (Table 5)
At the end of the germination test, remaining seed should be cut and examined. The seed is cut open and internal structures are observed. Fully developed, firm tissue with the proper coloring is considered viable while shriveled, decayed and discolored tissue or seed lacking an embryo is considered nonviable. If the seed has not been prechilled and an extremely high percent of viable seed is found, a retest of prechilled seeds may be advisable.

(6) Hard Seeds - The percentage of hard seed is to be reported in addition to the percentage germination. If swollen seeds or seeds which have started to germinate are present at the end of the prescribed germination period, remove all hard seeds (record their number) and continue the swollen seed in test for 14 days, when germinating at 15-25^C or 10 days when germination at 20^C for species listed in Table 3 and continue the test for 5 additional days for species listed in Table 4. The additional normal seedlings shall be included in the percentage of germination.

For hard seeds in tree and shrub seed (Table 5) see 4.9k (3).

Other changes

4.2e. adjustment detailed in proposal 11.
4.6b. delete reference "..., such as the presence of firm ungerminated seeds, ...".
4.9d. (second to last paragraph beginning with "If at the end...) Some coordination between 4.9d section (proposal number 12) and 4.9k (6) will need to be done if both proposals are accepted.
4.9d. (last paragraph) delete in this position and transfer concept to 4.9k (5) or retain and designate this paragraph as 4.9d (7).

Table 3 footnotes (page 58)

a. replace "see sections 4.2e and 4.9" with "see sections 4.2dand 4.9k (6) ".

d. replace "see section 4.2e" with "see sections 4.2e and 4.9k".

e. replace text following "Hard seeds often present " with "see section 4.9k (6)".

Table 4 footnotes (page 74)

b. (reword as indicated - new is underlined, deletion cross-out.)"Final count may vary with certain types, cultivars, or strains within any flower seed kind. Remaining seeds at the end of test should be critically examined for any ~~fresh, firm,~~ viable seeds and ~~either~~ recorded as ~~firm~~ dormant seeds, ~~held in test longer, or retested and given special treatment to stimulate these sensitive seeds to germinate."~~
or,
replace text with "see 4.9k".
or,
delete altogether

c. replace text following "Hard seeds often present" with "see 4.2d and 4.9 k (6)".

d. replace textwith " Embryo excision method: see 4.9k (1)".

Table 5 footnotes (page 84)

a. Replace text with "Embryo excision method: see 4.9k (1)".

b. Replace text with "TZ tetrazolium : see 4.9k (2)".

c. Replace text with "Hard seed often present: see 4.9k (6)".

REASONS FOR PROPOSAL

A clear destinction must be made between methods for determining germination and methods for determining dormancy. Much confusion is caused by not having methods for determining viability of ungerminated seed at the end of the test in one central location. A new section 4.9k is proposed for this purpose. This centralization will hopefully bring to attention conflicting procedures listed in different locations such as illustrated by the hard seed instruction at the end of Tables 3,4, and 5. Centralizing will also permit more detailed description of methods and listing of references.

There are some procedures listed in 4.8a, b and d for dealing with dormant seeds but it is not specified if the seedlings derived from the used of the procedures or to be listed as germinated or dormant. There is also some confusion about the categorizing seeds of tree and shrub species which are designated as viable by designated as viable by the cutting test. We are proposing that they be designated as dormant. This proposal also paves the way to simplifying and clarifing the reorganization of the flower seed germination procedures in Table 4 which is now underway by the Flower Seed Committee.

SUBMITTED BY:

Rules Committee and Flower Seed Committee of AOSA

14. KIND OF SEED

Glycine max - soybean

PRESENT RULE

In Table 3 Methods of testing for laboratory germination, Agricultural seeds, The substrata listed for Glycine max are B,T,S, and TC. In the same table but in the Vegetable and Herb section the substrata listed for G.max has TC omitted.

PROPOSED RULE

In Table 3 section Vegetable and Herb seeds, add TC to the substrata list for G. max to conform to the list for the same species in the Agricultural seeds section.

SUPPORTING EVIDENCE

The substrata TC was accepted for G. Max in 1980. There is no evidence that the substrata for the same species should be different for the two listings in Table 3.

SUBMITTED BY:

Steve McGuire - Michigan

DATE:

October 30, 1984

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15. SUBJECT

Photographs of seedlings references

PRESENT RULE:

4.9j footnote a:These photographs may be purchased from the Office of Information, United States Department of Agriculture, Washington, D.C. 20250.

PROPOSED RULE

4.9J footnote a: Only the photographs described in the AOSA Newsletter 57(3):67-72 (September 1983) may be purchased from the Office of Information, United States Department of Agriculture, Washington, D.C. 20250.

REASON FOR THE PROPOSED CHANGE

Many of the photos listed in Table 3 are no longer available. This is due to the poor quality of some photos and the deterioration of certain negatives. The Federal Seed Laboratory staff has reviewed the photos and negatives and determined which photos are still suitable. Information pertaining to the photos that are still available was printed in the AOSA newsletter, Vol. 57, No. 3, Pg. 67-72 (September 1983) and on page 39 of the September 1983 issue of the Seed Technologist News.

SUBMITTED BY

Richard C. Payne, Supervisor, Federal Seed Laboratory, Building 306, Room 213, BARC-East, Beltsville, MD 20705

DATE:

November 23, 1984

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16. KIND OF SEED
Lettuce (Lactuca sativa)

PRESENT RULE
Appendix 1. Seedling Descriptions
Section, Asteracae, Sunflower family
Section A. Lactuca Sativa (lettuce), page 106 of the AOSA Rules

PROPOSED RULE
Appendix 1, Seedling Descriptions, for lettuce should be amended as follows:
2. Asteraceae, Sunflower family
A. Lactuca sativa, lettuce

One type of necrosis on lettuce cotyledons appears to be a physiological breakdown of the plant tissues, the cause of which has not been determined but appears to be associated with age of the seed. 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 age. proximity to light, delayed germination or dormancy.

Colored photographs and an interpretive drawing are available from the Federal Seed Laboratory, Beltsville Maryland, and should be used as guides to classification of lettuce seedlings. It is not necessary to distinguish physiological necrosis from other types of necrosis since the interpretation is the same for all. Magnification up to 7X may be used for interpretation. Seed coats may have to be removed for seedling evaluation.

The following interpretations are to be made only at the end of the test period:

Normal seedling

Root	Strong primary root, usually with root hairs and with no splits or lesions.
Hypocotyl	Strong, with no cracks or lesions extending into the conductive tissues.
Cotyledons	(a) two (b) if necrosis or injury is present, classify as normal if the necrosis or injury covers less than half the total cotyledon area.
Epicotyl	Present and entirely free from necrosis or decay. (May be assumed to present if cotyledons are normal.

Abnormal seedling

Root	(a) None (b) tips blunt, swollen, discolored (c) splits or lesions.
Hypocotyl	Severely twisted, grainy or with cracks or lesions extending into the conductive tissue.
Cotyledons	(a) only one (b) necrosis or injury covering one half or more of the total cotyledon area. (c) Swollen cotyledons usually associated with extremely short or vestigial hypocotyl and root.
Epicotyl	Missing or with any degree of decay.

SUPPORTING EVIDENCE OR REASONS FOR THE PROPOSED CHANGE

This is a proposal for rewriting section 2A of Appendix 1 - Seedling descriptions. The purpose is to eliminate all references to hypocotyl and root length as they pertain to normal and abnormal seedlings. It is also worded to eliminate references to color of physiological necrosis on lettuce cotyledons. It eliminates the need to keep reference samples of all lettuce cultivars. This proposal also removes the reference to Handbook 30 which is no longer available and in which the descriptions are inadequate.

JUSTIFICATION

Paragraph one of the present rules defines "normal length" as "the length attained by a vigorous sample of the same kind and cultivar when grown under the exact test conditions as the sample in question." Every major vegetable seed producer probably has their own cultivars, and introduces new ones continously. It is impossible to maintain "vigorous" reference samples of all known cultivars of lettuce currently on the market. Therefore it is necessary to eliminate that requirement.

The principal causes of variation between laboratories testing the same samples appear to be differences of opinion on what constitutes physiological necrosis (color interpretation), and/or "normal length."

The present rules describe physiological necrosis as "softened, grayish, reddish or blackish." There is no mention of other colors such as yellow, green, or white. The word discolored in the new proposal would eliminate color references and allow comparison with healthy cotyledons of the same replicate. Color can vary considerably depending on the amount of chlorophyll in the cotyledon; which in turn is dependant on test conditions and proximity and intensity of the lights in the germinator.

Hypocotyl and root length can vary according to the age of the seedling. In some samples there appears to be a bit of residual dormancy which causes some seedlings to germinate later in the test period. A seedling which germinates on the fifth day of the test will not reach "½ normal length" of a seedling that had germinated on the second day. Some cultivars which are extremely light sensitive may show delayed germination and therefore fewer seedlings would reach "normal length." Such seedlings would then be condidered abnormal by strict adherance to the rule.

Hypocotyl length can vary somewhat with the paper media used for the test even when all other conditions are the same. Following is a summary of measurements made of a single cultivar grown on different media in plastic boxes;

4 layers filter paper	- 17.9 mm
2 layers blue blotters	- 23.9 mm
3 layers brown paper towel	- 25.0 mm

In itself this is probably not enough to cause a difference of interpretation, but combined with other factors could be significant. The difference can probably be attributed to the water holding capacity of the different media, and possibly the light reflective qualities of the different surface textures and colors of paper.

Hypocotyl length can vary according to seed lot as illustrated by the following measurements of two seed lots of each of three cultivars:

Cultivar	Seed Lot 1	Seed Lot 2
Black-seeded Simpson	20.8 mm	21.5 mm
Parris Island	19.8 mm	26.0 mm
Oak Leaf	15.4 mm	19.3 mm

This would indicate that reliance on a reference sample might be misleading.

Hypocotyl length can be influenced by light intensity. Seedlings growing under high light intensity are shorter and stronger than those growing under weak light. Distance from the light source determines the intensity of light reaching the seedlings. Therefore, distance from the light source causes a difference in hypocotyl length as shown in the following summary of measurements taken of one cultivar at different positions in the germinator

Distance from light (inches)	2	6	10	14
Average Hypocotyl length (mm)	6.2	13.2	17.8	22.6
Range in length (mm)	4-8	11-15	15-20	20-28

Thus some seedlings grown nearest the lights are less than ¼ the length of those grown furthest from the light. The range of individual seedling lengths was from 4 mm. to 28 mm. within the same seed lot. Light intensity varied from 1400 to 2600 lux.

Length can vary according to a combination of factors such as cultivar, position in the germinator, germination chamber, and intensity of light. Two experiments were conducted, using different germinators and several cultivars. Lights were at the back only and the walls of both germinators were painted white. The results are summarized as follows:

Germinator I	distance from light (inches)			
(light 1400-2600 lux)	2	6	10	14
Parris Island (avg. length mm)	19.36	28.76	30.60	32.56
Oak Leaf	10.04	21.68	26.68	28.52
Salad Bowl	18.20	26.92	33.20	32.92
Black seeded Simpson	14.96	25.16	27.04	28.08
Average all cultivars:	15.64	25.63	29.38	30.52
Germinator II				
(light 2000-3000 lux)				
Parris Island (avg. length mm)	14.35			25.15
Oak Leaf	9.10			21.75
Black-seeded Simpson	17.15			24.40
Iceberg	9.2			23.10
Grand Rapids	13.75			31.54
Average all cultivars:	12.71			25.19

Some cultivars appear to be more responsive to light intensity than others. In some instances (Oak Leaf, Iceberg) the differences would be enough to cause some seedlings to be considered abnormal (that is less ½ normal length). Entire replicates or tests could be misread if they should be growing nearer the lights than their corresponding check samples.

There appears to be a similar but reverse reaction in root length. Precise measurements were not made, but it appears that the ratio of root to hypocotyl was greater for seedlings growing nearest the light source. In other words, roots were longer on seedlings at 2 inches from the lights as compared to those at 14 inches. Furthermore, roots

tended to grow in a direction away from the lights at a rate commensurate with light intensity. There was no direct correlation of root length and hypocotyl length. The opposite seemed to be the rule--seedlings near the lights had the shortest hypocotyls, but the longest roots. Therefore, it cannot be said that root and hypocotyl should be in proportion to total seedling growth.

Age of the seed can have an effect on hypocotyl and root length. Normal seedlings from old seed often have shorter hypocotyls and roots due to poor vitality. Since old seed often exhibits physiological necrosis, this is probably how physiological necrosis become associated with short hypocotyls and roots.

The 1954 Rules (Vol. 44, No. 2) were printed without seedling descriptions. That same year, the recommendations of the Subcommitttee for Evaluation of Lettuce Seedlings (Vol. 44, 1954 Proc. AOSA, page 26) and the Standardized Test Committee (Vol. 45, 1955 Proc. AOSA page 28) clearly state that "seedlings with roots less than half normal length are acceptable as normal sprouts, provided root hairs are present, the root tips are not dead and the hypocotyls and cotyledons are normal as described herein."

Likewise, "seedlings with hypocotyls of less than half the normal length are acceptable as normal sprouts provided the roots and cotyledons are normal as described herein."

Between the time of the committee reports and the next printing of the rules in 1960 (Vol 49, No. 2, Proc. AOSA) the wording was changed to "preferably over half normal length" for both hypocotyl and root. This is being misinterpreted as a specific requirement for all normal seedlings.

There does not appear to be any valid reason to associate physiological necrosis with root and hypocotyl length. Physiological necrosis may be observed on seedlings of any length. Therefore, the two conditions should not be linked.

Variations in test results arise because some analysts feel that seedlings exhibiting physiological necrosis must also be less than ½ normal length to be considered abnormal. Some analysts consider any seedling less than ½ normal length to be abnormal regardless of cotyledon condition. Others evaluate physiological necrosis apart from length of hypocotyl and root and classify necrotic seedlings on the basis of total cotyledon area (necrosis covering ½ area or more is considered abnormal). Because hypocotyl and root length can be affected by many factors not related to necrosis, and because we cannot be sure what "normal length" should be, the description of "normal length" should be dropped.Hypocotyl and root length should not be used as criteria for judging seedlings with physiological necrosis. In effect, this would eliminate the need to maintain reference samples.

This brings the rule back to one simple consideration for necrosis of any sort. Any necrosis less than ½ the total cotyledon area would be normal; necrosis covering ½ or more of the total cotyledon area would be abnormal. If this proposal were to be adopted, it would simplify lettuce seedling evaluation and possibly bring laboratories into closer agreement on test results. It would also bring the AOSA rules into agreement with the current ISTA rules with regards to lettuce germination.

One final note: split roots are not presently covered by the seedling descriptions. Therefore, it has been written into the proposed rule.

SUBMITTED BY

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DATE:

November 23, 1984

AOSA Rules Committee:

1985 Arnold L. Larsen (Chairman)
1986 Ellen M. Chirco
1987 Stan Kirkland
1988 Allen Knapp
1989 Stephen J. Hurst

Ex-officio members:

Robert W. Yaklich (Editorial)
Wayne R. Guerke (Research)
Mark W. Johnson (SCST)
Ellen M. Chirco (Referee)

ANNOUNCEMENTS AND REPORTS

Report and Announcement of the Seed Sample Mediation Subcommittee of the Seed Standardization Committee

Chairman - Gail Fenderson, Plant Industry Supervisor, OK State Department of Agriculture, 2800 N. Lincoln Boulevard, Oklahoma City, OK 73105
Members - Larry Nees, Indiana and Hollis Buckland, Vermont

If you have read the recent September AOSA News Letter you will note the Seed Standardization Committee is now an official standing committee of AOSA. On pages 60 and 61 of the News Letter there is an outline of the Purpose and Operation of the Seed Sample Mediation Subcommittee. Although the Executive Board has not set a sample fee to cover costs for shipping samples and postage for reporting results of test, we are announcing and encouraging AOSA laboratories to send samples to us where there is a disagreement between analysis results on an official test and any other laboratory test.

Samples submitted to the mediation committee must be of sufficient size to obtain at least three working weights for the particular analytical problem in question, At least two AOSA cooperating laboratories will be selected to run the appropriate analysis. A survey of laboratories has been made, so we already have a list of laboratories for each kind of seed.

We are not hoping you have a sample problem, but if you do, forward the sample to the above address. We do need a few samples to establish a cost basis, as well as a handling and reporting procedure.