RULES PROPOSALS 1991

AOSA Rules Committee
David F. Svik, Chairman

The following proposals for changes in or additions to the AOSA Rules for Testing Seeds have been reviewed and approved by the Rules Committee for further consideration by the AOSA members at the 1991 June meeting. Approval does not mean that the Committee or the members endorse these proposals to the Rules.

Eighteen proposals are presented as required by the Constitution so the membership can review them 90 days prior to the 1991 AOSA meeting. Please evaluate these proposals carefully. The name and address of the author(s) are noted if you wish to contact them for additional information or have comments.

Comments concerning any of these proposals should be made in writing to the Rules Chairman before the June meeting. Additional comment time will be available during the Open Rules meeting held prior to the AOSA business meeting in June. Extensive changes to the proposals are possible at the Open Rules meeting but are not encouraged by the Rules Committee.

Please Note: Only a limited number of copies of these proposals will be available at the Open Rules meeting. We recommend that you bring your copy of this Newsletter with you to the Open Rules meeting.

1. PROPOSAL

Reduce final count to 14 days for sand dropseed (Sporobolus cryptandrus) in Table 3.

PRESENT RULE

				Additional Directions	
Substrata	Temp C	First Count Days	Final Count Days	Specific requirements	Fresh and dormant seed
P	5-35; 15-35	7	28[d]	Light, KNO_3	Prechill at 5 C for 4 weeks

PROPOSED RULE

				Additional Directions	
Substrata	Temp C	First Count Days	Final Count Days	Specific requirements	Fresh and dormant seed
P	5-35; 15-35	7	14[d]	Light, KNO_3	Prechill at 5 C for 4 weeks

SUPPORTING EVIDENCE

Attached.

SUBMITTED BY

Rangegrass Analysis Subcommittee
Tim Gutormson, Chairman,
Phone: 515-294-6826

The proposed change would reduce the germination test length by 14 days. Data collected from laboratories on sand dropseed germination are presented in Table 1, 2, and 3.

Data presented in Table 1 represents 70 seed lots which received 28 days of prechill followed by germination testing up to 28 days. Germination in the first 14 days of testing equaled 52.8% compared to 1.2% additional germination in the last 14 days of testing. These data indicate that the germination testing period could be shortened to 14 days without greatly increasing dormant seed percentages.

Table 2 data represents 31 seed lots which received 14 days of prechill followed by up to 28 days of germination testing. Germination in the first 14 days of testing equaled 63.5% compared to 0.1% additional germination in the last 14 days of testing. Shortening the germination test to 14 days on these seed lots would not significantly increase seed dormancy.

Data presented in Table 3 represents 25 seed lots which did not receive a prechill treatment and were germination tested for 28 days. Germination in the first 14 days of testing equaled 16% compared to 1% additional germination in the last 14 days of testing. Reducing the germination test to 14 days on these seed lots would not change dormancy levels greatly.

The six laboratories submitting data used four different temperatures when germinating sand dropseed. Three of these temperatures are not recommended in the A.O.S.A. Rules, however germination percentages and speed of germination were comparable to the recommended temperature. Further investigations on the optimum germination temperature for sand dropseed appear warranted, but are beyond the intended scope of this study.

Table 1. Sand dropseed viability data collected from seven seed laboratories representing 70 seed samples tested from 1987 to 1990 which were prechilled for 28 days, germinated at either 15/30, 15/35, or 20/35 C with light and 0.2% KNO_3.

LAB NO.	YEAR(s)	DAYS PC	GERMINATION COUNTS ---days--- 7	14	21	28	TOTAL	DORMANT	GERM + DORM	DAYS TESTED	NO. OF SEED-LOTS
			%								
6.	87-90	28	52	1	1	1	54	31	85	56	13
1.	87-89	28	39	1	0	0	40	47	87	56	25
3.	88-90	28	79	5	0	0	84	10	94	56	4
2.	87-90	28	60	3	-	-	63	25	88	42	10
2.	87-90	28	41	15	8	-	64	7	71	49	6
4.	88-89	28	70	7	-	-	77	18	95	42	1
4.	87-88	28	59	1	-	1	61	30	91	56	11
AVERAGE/TOTAL		28	50	2.8	0.9	0.3	54	32.3	86.7	--	70

Table 2. Sand dropseed viability data collected from 2 seed laboratories representing 31 seed samples tested from 1988 to 1990 which were prechilled for 14 days, germinated at 15/25 and 15/30 C with light and 0.2% KNO_3.

LAB NO.	YEAR(s)	DAYS PC	GERMINATION COUNTS ---days--- 7	14	21	28	TOTAL	DORM-ANT	GERM + DORM	DAYS TESTED	NO. OF SEED-LOTS
			---%								
5.	1989	14	56	7	-	-	63	26	89	28	27
3.	88-90	14	57	10	1	0	69	26	94	42	4
AVERAGE/TOTAL		14	56.1	7.4	0.1	0	63.6	26	89.6	--	31

Table 3. Sand dropseed viability data collected from 1 seed laboratory representing 25 seed samples tested in 1989 which received no prechilled treatment and were germinated at 15/30 C with light and 0.2% KNO_3.

LAB NO.	YEAR(s)	DAYS PC	GERMINATION COUNTS ---days--- 7	14	21	28	TOTAL	DORM-ANT	GERM + DORM	DAYS TESTED	NO. OF SEED-LOTS
			---%								
1.	1989	0	14	2	1	0	17	78	95	28	25
AVERAGE/TOTAL		0	14	2	1	0	17	78	95	28	25

Laboratories submitting data for this proposal.

Lab. No.	Laboratory Name
1.	Arkansas Valley Seed Co. (A.V. Seeds, Inc.)
2.	Lubbock Seed Laboratory, Texas Dept of Ag.
3.	Nebraska State Seed Testing Laboratory.
4.	Pieratt Seed Laboratory, Giddings TX, Texas Dept. of Ag.
5.	South Dakota State Seed Testing Laboratory
6.	Stephenville Seed Laboratory, Texas Dept. of Ag.

2. PROPOSAL

Reduce final count to 14 days for yellow bluestem (Bothriochola ischaemum) in Table 3.

PRESENT RULE

				Additional Directions	
Substrata	Temp C	First Count Days	Final Count Days	Specific requirements	Fresh and dormant seed
P,TS	20-30	7	21[d]	Light, KNO_3	Prechill at 5 C for 2 weeks

PROPOSED RULE

				Additional Directions	
Substrata	Temp C	First Count Days	Final Count Days	Specific requirements	Fresh and dormant seed
P,TS	20-30	7	14[d]	Light, KNO_3	Prechill at 5 C for 2 weeks

SUPPORTING EVIDENCE

Attached.

SUBMITTED BY

Rangegrass Analysis Subcommittee
Tim Gutormson, Chairman,
Phone: 515-294-6826

The proposed change would reduce the germination test length by 7 days. Data collected from laboratories on yellow bluestem germination are presented in Table 1.

Data presented in Table 1 represents 344 seed lots which received 14 days of prechill followed by germination testing up to 28 days. Germination in the first 14 days of testing equaled 60.6% compared to 6.9% additional germination in the last 14 days of testing. These data indicate that the germination testing period could be shortened to 14 days without greatly increasing dormant seed percentages.

Table 1. Yellow bluestem viability data collected from seven seed laboratories representing 334 seed samples tested from 1985 to 1990 which were prechilled for 14 days, germinated at 20/30 C with light and 0.2% KNO_3.

LAB NO.	YEAR(s)	DAYS PC	GERMINATION COUNTS ---------days--------- 7	14	21	28	TOTAL	DORM-ANT	GERM + DORM	DAYS TESTED	NO. OF SEED-LOTS
			----------%								
3.	89-90	14	54	4	-	-	58	5	63	28	14
2.	87-90	14	68	1	4	-	73	1	74	35	6
2.	87-90	14	66	7	-	-	73	1	74	28	59
4.	87-90	14	55	3	1	-	59	0	59	35	92
1.	86-89	14	65	6	1	-	72	1	73	35	10
3.	85-88	14	62	5	4	0	71	0	71	42	153
AVERAGE/TOTAL		14	60.6	4.7	2.2	0	67.5	0.4	67.9	--	334

Laboratories submitting data for this proposal.

Lab. No.	Laboratory Name
1.	Colorado Seed Testing Laboratory
2.	Lubbock Seed Laboratory, Texas Dept of Ag.
3.	Pieratt Seed Laboratory, Giddings TX, Texas Dept. of Ag.
4.	Stephenville Seed Laboratory, Texas Dept. of Ag.

RULES PROPOSAL No. 3

Addition of "P" substrata to germination prescription for Lotus corniculatus (Birdsfoot trefoil) in table 3.

PRESENT RULE

Kind of Seed	Substrata	Temperature °C	First Count	Final Count
Lotus corniculatus birdsfoot trefoil	B,T	20	5	12[a]

PROPOSED RULE

Kind of Seed	Substrata	Temperature °C	First Count	Final Count
Lotus corniculatus birdsfoot trefoil	B,T,P	20	5	12[a]

SUPPORTING EVIDENCE

Data collected from 11 laboratories on 3 lots of seed (Table 1) indicated that substrata "P" produced germination results equivalent to those produced by substrata T & B.

Analysts observed that evaluation of abnormals on substrata "P" was very clear cut.

TABLE 1 *

	LAB #	23	27	56	68	111	112	114	115	116	119	121	AVERAGE	STANDARD DEVIATION
SAMPLE 1	T	91	(86)	89	92	91	94	93	95	92	87	93	91.2	2.6
	B	95	95	91	95	93	94	96	94	93	92	94	93.8	1.5
	P	92	92	91	93	95	(98)	93	92	93	95	94	93.5	2.0
SAMPLE 2	T	(74)	83	76	77	76	82	86	83	87	(70)	83	79.7	5.3
	B	83	87	(75)	81	81	81	82	85	85	83	86	82.6	3.3
	P	81	77	(75)	84	87	84	84	82	82	83	86	82.3	3.6
SAMPLE 3	T	69	76	(64)	69	75	81	71	79	83	75	78	74.5	5.8
	B	70	79	68	72	70	76	73	77	77	72	(86)	74.5	5.1
	P	75	72	71	74	71	(84)	(66)	77	81	76	(86)	75.7	6.0

( ) = results out of tolerance at 5% level but were included in all calculations

* Data compiled by Seed Analysts of the Midwest referee

SUBMITTED BY

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DATE OF PROPOSAL

October 9, 1990

No. 4

Rules Proposal: Addition of Hilaria jamesii--galleta grass to the rules.

Present Rule: New Rule

Proposed Rule:

1. Include in Table 1 (Weights for working samples, AGRICULTURAL SEEDS) the following:

Kind of Seed	*Minimum weight for purity analysis*	*Minimum weight for noxious weed seed examination*	*Approximate number of seeds per gram*	*Approximate number of seeds per ounce*
	Grams	*Grams*	*Number*	*Number*
Hilaria jamesii (Torr.) Benth. galleta grass				
(other than caryopses)	*10*	*100*	*260*	*7400*
(caryopses)	*5*	*50*	*580*	*16500*

2. Add the phrase, "Spikelet groups that disarticulate as a unit in galleta grass (Hilaria jamesii)", at the beginning of 2.6(4).

3. Include in Table 3 (Methods of testing for laboratory germination, AGRICULTURAL SEEDS) the following:

Kind of Seed	*Substrata*	*Temperature C*	*First Count Days*	*Final Count Days*
Hilaria jamesii (Torr.) Benth. galleta grass	*P, B*	*20, 25, 20-30*	*4*	*10[d]*

d. Determine viability of ungerminated seeds. See section 4.9K.

Supporting Evidence:

1. The working weights listed are based on 2000-seed samples from four lots for naked caryopses and on 500-seed samples from 4 lots for spikelet groups. There was little between-lot variation.

2. Galleta grass is commonly marketed as 1-seeded spikelet groups but is occasionally marketed as threshed caryopses. Both seed unit types are defined as pure seed since both are easily tested for germination.

In a referee purity test on unthreshed seed, a light table/pressure technique for determining pure seed gave purity values between 68% and 78% for 6 of 7 participating laboratories.

3. Galleta grass is reported to show little or no dormancy, germinating rapidly and completely in the dark at temperatures from 20C to 40C (Knipe, 1967, 1968; West, 1972; and Sabo et al., 1979).

In a 4-source experiment at 20C including 1 fresh source, threshed seed showed no response to light, potassium nitrate, or GA3. All sources germinated to within 1% of total viable seed within 7 days. In an experiment with unthreshed seed at 20C in the dark, germination was also essentially complete in 7 days. A 10-day test period is recommended to allow normal and abnormal seedling classification. Since galleta is sometimes collected from native stands with unknown dormancy characteristics, a viability determination on ungerminated seed is recommended.

In an 8-laboratory referee test on unthreshed seed, 4 laboratories had high values (84-87%) which did not differ significantly, while 3 had somewhat lower values (66-74%). Some of the variation in germination was compensated for by variation in percent purity, with pure live seed values ranging from 50% to 61% for 7 laboratories. This suggests that the light table/pressure method for purity

analysis is subject to some error with respect to the selection of filled spikelet groups, a problem that is common with some other grasses.

Literature Cited:

Knipe, O. D. 1967. Influence of temperature on the germination of some range grasses. J. Range Manage. 20: 298-299.

Knipe, O. D. 1968. Effects of moisture stress on germination of alkali sacaton, galleta, and blue grama. J. Range Manage. 21: 3-4.

Sabo, D. G., G. V. Johnson, W. C. Martin, and E. F. Aldon. 1973. Germination requirements of 19 species of arid land plants. USDA. USFS Research Paper RM-210. 26 pp.

West, N. E. 1972. Galleta: taxonomy, ecology and management of Hilaria jamesii on western rangelands. Utah Agric. Expt. Sta. Bull. 487. 38 pp.

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Date of Proposal:
November 6, 1990

Rules Proposal No. 5

KIND OF SEED (Scientific and common name):

Phaseolus vulgaris Garden bean

PRESENT RULE

4.8 j.

Garden bean (Phaseolus vulgaris).- Use of calcium nitrate: 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.

PROPOSED RULE

4.8 j.

Garden bean (Phaseolus vulgaris).- Use of calcium nitrate: If hypocotyl collar rot is observed on seedlings, the sample involved shall be retested using a 0.3 to 0.6 percent calcium nitrate solution to presoak the substratum.

SUPPORTING EVIDENCE OR REASONS FOR THE PROPOSED RULE:

The proposal is to change the word may to shall. The word may gives permission, however it does not give direction. In the introduction of the AOSA Rules For Testing Seeds, it states that the procedures specified in the rules are to be followed as a matter of routine. Routine is defined by The American Heritage Dictionary Second College Edition as a prescribed and detailed course of action to be followed regularly, therefore the word shall should be used to give direction to the rule.

*Attached literature clearly indicates that seed lots of garden bean exhibiting symptoms of hypocotyl collar rot demonstrate a significant increase in the percentage of normal seedlings when the substratum is presoaked in a solution of 0.3 to 0.6 percent calcium nitrate.

To promote uniformity between sections 4.3, 4.9, and tables 3,4,5, substratum is substituted for medium.

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uniformity subcommittee

11/14/90

* Information noted above is available from author or any Rules Committee

Rules Proposal No. 6

KIND OF SEED:

Lolium X hybridium, Haussknecht, intermediate ryegrass

PRESENT RULE:

New Rule

PROPOSED RULE:

1) Include in Table 1 (Weights for working samples, Agricultural Seeds) the following:

Kind of seed	Min.wt.for purity anal, (g)	Min.wt.for noxious-weed seed exam. (g)	Approx. no. seeds/gram	Approx. no. seeds/oz.
Lolium x hybridum Haussknecht intermediate ryegrass	8	80	338	9580

2) Include in Table 3 (Methods of testing for laboratory germination, Agricultural Seeds) the following:

Kind of seed	Subs.	°C Temp.	First count days	Final count days	Spec. req.	Fresh and dormant seed
Lolium x hybridum Haussknecht intermediate ryegrass	P,TB	15-25	7	14	Light	KNO3 and prechill at 5°C or 10°C for 5 days and test at 15-25°C; if necessary re-chill for 3 days and continue test at 15-25°C an additional 4 days.

SUPPORTING EVIDENCE OR REASONS FOR THE PROPOSED RULE:

Germination tests on seven lots were conducted by five laboratories using 15-25°C. Four laboratories also tested these lots using 20-30°C. Results obtained were very similar for both temperature combinations. The 15-25°C temperature is recommended so as to be consistent with that specified for other Lolium spp., since mixtures of Lolium x hybridum and other Lolium species often need to be tested.

15-25°C
Laboratory

Sample#	#1	#2	#3	#4	#5
60178	93	91	93	94	94
61156	95	93	95	95	97
63924	98	96	94	96	97
63997	97	95	95	95	95
68222	97	95	94	97	97
68223	94	90	95	95	95
68861	96	97	91	91	93

20-30°C

60178	--	93	95	90	94
61156	--	93	91	95	98
63924	--	95	96	95	98
63997	--	95	94	95	96
68222	--	97	95	94	96
68223	--	89	95	95	98
68861	--	96	91	90	92

Seed counts were conducted on each of the seven lots by two laboratories. The average of the highest and lowest figures from each laboratory were in turn averaged to obtain the number of seeds per gram. This value was multiplied by 28.35 to determine the number of seeds per pound.

SUBMITTED BY:

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DATE: 11/13/90

RULES PROPOSAL No. 7

Fluorescence test of ryegrass

PRESENT RULE:

3.5 Fluorescence test of ryegrass. — A fluorescence test shall be made on all samples of ryegrass for which the proportions of the perennial ryegrass (*Lolium perenne*) and annual or Italian ryegrass (*L. multiflorum*) is to be determined. The seedlings shall be grown on filter paper and the number of fluorescent seedlings determined under ultraviolet light at the end of the germination period. Percentages of pure seed, fluorescence, nonfluorescence, and germination shall be determined and the results shall be employed to calculate the proportion of annual and perennial ryegrass present in the sample with formula 1 or 2 below.*

a. *Formula 1.* — If more than 75% of the normal seedlings are fluorescent, use the following formula:

$$\% \text{ annual or Italian ryegrass} = \frac{\% \text{ fluorescent seedlings} \times \% \text{ pure ryegrass}}{\% \text{ germination}}$$

% perennial ryegrass = % pure ryegrass less the % annual or Italian ryegrass

Example: Pure ryegrass = 99.0%
Fluorescence = 82% (328 normal fluorescent seedlings divided by 400)
Nonfluorescence = 3% (12 normal nonfluorescent seedlings divided by 400)
Germination = 85% (340 total normal seedlings divided by 400)

$\frac{328}{340}$ = 96.5%, percentage normal seedlings which are fluorescent. It is higher than 75%, therefore, formula 1 applies.

Substituting: $\frac{82\% \times 99\%}{85\%}$ = 95.51% annual or Italian ryegrass

99.0% - 95.51% = 3.49% perennial ryegrass

b. *Formula 2.* — If less than 75% of the normal seedlings are fluorescent, use the following formula:

$$\% \text{ perennial ryegrass} = \frac{1.05 \times \% \text{ nonfluorescent seedlings} \times \% \text{ pure ryegrass}}{\% \text{ germination}}$$

% annual or Italian ryegrass = % pure ryegrass less the % perennial ryegrass

Example: Pure ryegrass = 98.50%
Fluorescence = 16.5% (66 normal fluorescent seedlings divided by 400)
Nonfluorescence = 73.5% (294 normal nonfluorescent seedlings divided by 400)
Germination = 90% (360 total normal seedlings divided by 400)

$\frac{66}{360}$ = 18.3%, percentage normal seedlings which are fluorescent. It is less than 75%, therefore, formula 2 applies.

Substituting: $\frac{1.05 \times 73.5\% \times 98.5\%}{90\%}$ = 84.46% perennial ryegrass

98.5% – 84.46% = 14.04% annual or Italian ryegrass.

*For description of method and apparatus for determining fluorescence in ryegrass see the article in the AOSA Newsletter 37 (3): 20-27, 1963. The factor 1.05 is to be used instead of 1.0526 as given in the article cited.

PROPOSED RULE:

3.5 Fluorescence test of ryegrass.--A fluorescence test shall be made on all samples of ryegrass for which the proportion of perennial ryegrass (Lolium perenne) and annual or Italian ryegrass (L. multiflorum) is to be determined. The seedlings shall be grown on filter paper and the number of fluorescent seedlings determined under ultraviolet light at the end of the germination period. Fluorescence results are to be reported as true fluorescence (T Fl) determined as follows:

$$\frac{\text{Number of normal fluorescent seedlings}}{\text{Total number of normal seedlings}} \times 100 = \text{T Fl}$$

The percentage of true fluorescence shall be modified by the fluorescence level of the variety being tested (V Fl) and then applied to the percentage of pure ryegrass from the purity test in Formula 1. or 2. below, depending on the kind of ryegrass being tested.[a]

A list of fluorescence level descriptions for ryegrass varieties is maintained by the Grass Variety Review Board of The Association of Official Seed Certifying Agencies. If the variety being tested is not stated or has not been described, V Fl in the perennial formula and V N Fl in the annual formula shall be considered to be zero. In blended mixtures, the factor shall be interpolated according to the proportion of each variety in the blend.

a. Formula 1.--Perennial Ryegrass:

$$\frac{\%\ \text{T Fl} - \%\ \text{V Fl}}{100} \times \%\ \text{pure ryegrass} = \%\ \text{annual ryegrass}$$

$$\%\ \text{pure ryegrass} - \%\ \text{annual ryegrass} = \%\ \text{perennial ryegrass}$$

Example (a): True Fluorescence = 1.88%
Variety not described
Pure ryegrass = 98.56%

Substituting: $\frac{1.88\% - 0.00\%}{100} \times 98.56\% = 1.85\%$ annual ryegrass

$98.56\% - 1.85\% = 96.71\%$ perennial ryegrass

Example (b): True Fluorescence = 1.88%
Varietal Fluorescence description=1.50%
Pure ryegrass = 98.56%

Substituting: $\frac{1.88\% - 1.50\%}{100} \times 98.56\% = 0.37\%$ annual ryegrass

$98.56\% - 0.37\% = 98.19\%$ perennial ryegrass

If the True Fluorescence result is less than the level described for the variety, it is not necessary to apply the formula. All pure ryegrass shall be considered to be perennial.

b. Formula 2.--Annual Ryegrass

$$\frac{\%\ T\ Fl + \%\ V\ N\ Fl}{100} \times \%\ \text{pure ryegrass} = \%\ \text{annual ryegrass}$$

$$\%\ \text{pure ryegrass} - \%\ \text{annual ryegrass} = \%\ \text{perennial ryegrass}$$

$$VN\ Fl = 100 - V\ Fl$$

Example (a): True Fluorescence - 96.06%
Varietal Fluorescence description = 100%
V N Fl = 0.00%
Pure ryegrass = 99.23%

Substituting: $\frac{96.06\% + 0.00\%}{100} \times 99.23\% = 95.32\%$ annual ryegrass

$99.23\% - 95.32\% = 3.91\%$ perennial ryegrass

Example (b): True Fluorescence = 84.72%
Varietal Fluorescence description = 90%
V N Fl = 10%
Pure ryegrass - 98.41%

Substituting: $\frac{84.72\% + 10.00\%}{100} \times 98.41\% = 93.21\%$ annual ryegrass

$98.41\% - 93.21\% = 5.20\%$ perennial ryegrass

If the True Fluorescence result is greater than the level described for the variety, it is not necessary to apply the formula. All pure ryegrass shall be considered to be annual.

[a]For description of method and apparatus for determining fluorescence in ryegrass see the article in the AOSA Newsletter 37 (3) :20-27, 1963. The formula appearing above is to be used instead of the one given in the article cited.

SUPPORTING EVIDENCE OR REASONS FOR THE PROPOSED RULE:

Research has shown that there is no genetic linkage between fluorescence and the annual or perennial habit of Lolium. (Attachment A.) For many years there have been a few Lolium varieties which did not quite fit the assumption that annual ryegrass is 100% fluorescent and that perennial does not fluoresce. The original AOSA formula for perennial ryegrass included a 1.05+% factor to accommodate the early variety Linn just for that reason.

Recently an increasing number of ryegrass varieties with varying levels of fluorescence have been developed and are being marketed. Using the fluorescence test and grow-out procedures, fluorescence levels of varieties which are neither 0% (perennial) or 100% (annual) are being described and published so that they can be tested and labeled by a standard procedure. Therefore it becomes necessary to have AOSA testing rules and formulas which will be flexible enough to accommodate any fluorescence level.

Using the "1.05" formula by substituting other fluorescence levels for the .05 produced erroneous results. (Attachment B.) Therefore a new set of formulas have been developed.

Reporting True Fluorescence (Fl/germ) rather than the present method (Fl/400) is also a new concept. True Fluorescence presents a more accurate representation of actual fluorescence content for purposes of assessing and comparing test results, and it eliminates one step in applying a formula.

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Attachment A.

Relevance of the Fluorescence Test for Ryegrass

The Test

The fluorescence test for ryegrass had its beginnings in Germany in 1928 when Gentner (1928, 1929) reported that the roots of annual Italian ryegrass fluoresced under ultraviolet light, while perennial English ryegrass did not. The roots of the so-called fluorescent seedlings do not themselves fluoresce, but rather, the fluorescence is caused by a substance which diffuses from the roots into the filter paper where it is concentrated. The blue fluorescence does not appear when roots are grown on glass or unglazed pottery (Gentner, 1928). Axelrod and Belzile (1958) isolated the fluorescent compound, annuloline, by extracting the roots of annual ryegrass together with the paper on which they were grown. The compound is a weakly basic alkaloid with the formula $(C_{17}\ H_{10}\ ON\ (OCH_3)_3$ (Karimoto, Axelrod, Wolinsky, and Schall, 1962).

Gentner suggested using the fluorescence difference as a convenient method for identifying the two species. Several authors emphasized the importance and usefulness of Gentner's discovery (Linehan and Mercer, 1931; Foy, 1931; Chmelar, 1934; and Dorph-Petersen, 1934).

However, the fluorescence test soon received considerable criticism. As early as 1930, Nilsson (1933) reported results in disagreement with Gentner's assertion that perennial ryegrass did not show fluorescence. Later, Gentner himself recognized that exceptions can occur. Numerous workers reported that a low percentage of fluorescent seedlings was present in pure perennial varieties (Rampton, 1938); Baekgaard, 1955; and Sacks and Simon, 1960).

Many test factors affect the expression of fluorescence in laboratory tests. These include the filter paper (Schmidt, 1954), ultraviolet lamps (Patrick and Clark, 1962), light intensity (Gillet, 1961; Pierpoint, 1961), temperature (Kelly, 1971), and time in test (Schmidt, 1953). These factors have been standardized in the AOSA and ISTA Rules.

Inheritance of Fluorescence in Ryegrass

Several workers reported that the fluorescent reaction is controlled by a single dominant gene (Mercer and Linehan, 1931; Corkill, 1932; Trumble and Phipps, 1933; Woodforde, 1933, 1935; Linehan and Mercer, 1933). Nitzsche (1966) later discovered two gene pairs for fluorescence and two gene pairs for intensity of fluorescence.

Linehan and Mercer (1931), Dorph-Petersen (1934), and Woodforde (1949) showed there is no genetic linkage between fluorescence and the annual and perennial habit. In further substantiation of this, Nyquist (1963) developed a fluorescent perennial type, and Nitzsche (1963) developed a nonfluorescent annual type. However, Nyquist did not release his variety and suggested that, to maintain the usefulness of the fluorescence test, breeders should release only nonfluorescent perennial varieties.

Alternative Methods

A number of morphological characters have been used to distinguish annual and perennial ryegrass seeds and seedlings including:

marginal teeth of lemma and palea (Lakon, 1919)
leaf blades rolled or folded in young shoots (Rampton, 1938)
accelerated heading under continuous light (Nittler and Kenny, 1964)
leaf epidermis characters (Barros, 1975)

Techniques of starch and polyacrylimide gel electrophoresis using enzyme systems, proteins, and isoelectric focusing have resulted in successful separation of ryegrass varieties. Hayward and McAdam (1977), Neilson (1980), Ostergaard and Neilson (1981), and Gilliland et al. (1982) investigated differences in phosphoglucoisomerase enzyme systems in perennial ryegrass plants. Other enzymes in ryegrass plants including esterase, peroxidase and glutamate dehydrogenase were evaluated by Payne et al. (1980) and Payne and Koszykowski (1983). Seed protein differences were seen by Larsen (1966) and Nakamura (1979) using polyacrylamide gels and by DePrins and Van De Weghe (1983) using electrofocusing on polyacrylamide gels.

Ferguson and Grabe (1984) obtained distinctive protein banding patterns for annual and perennial ryegrass on an individual seed basis.

Perennial Ryegrass Fluorescence Calculations

Comparison of 1.xx formula and a proposed formula

The present perennial ryegrass formula is

$$\frac{1.05 \times \%\ \text{Non Fl} \times \%\ \text{PS}}{\%\ \text{Germ}} = \%\ \text{Perennial Ryegrass}$$

Since this formula was devised for Linn Perennial Ryegrass which was described as being 5% fluorescent, it was believed the same formula could be used for perennial ryegrass with any level of varietal fluorescence by substituting the described level for .05 in the formula. This does not work, as shown in the examples. In fact, since the 1.0526 was changed to 1.05 in the present formula, this formula is not accurate even for a variety with 5% fluorescence.

The proposed formula for perennial ryegrass is

$$\frac{\text{T Fl} - \text{V Fl}}{100} \times \text{PS} = \%\ \text{Annual Ryegrass}$$

The companion formula for annual ryegrass would be

$$\frac{\text{T Fl} + \text{V N Fl}}{100} \times \text{PS} = \%\ \text{Annual Ryegrass}$$

T Fl is Fl ÷ Germ. (It is being proposed that fluorescence test results always be reported as T Fl.)

V Fl is the fluorescence level as described for the variety.
V NFl is 100 - V Fl. (It is being proposed that all varieties of ryegrass, both perennial and annual, be described in terms of fluorescence content.)
If the variety being tested is not stated or has not been described, V Fl in the perennial formula and V N Fl in the annual formula would be considered to be zero.

Pure seed percentages resulting from application of present and proposed formulas to perennial ryegrass varieties with various fluorescence levels:

In all cases germination and pure ryegrass are both considered to be 100% in order to remove variability from those sources.

Example 1. Fluorescence (T Fl) results the same as described forthe variety:

		% Perennial		% Annual	
%V Fl	%T Fl	Present	Proposed	Present	Proposed
5	5	99.75	100.00	0.25	0.00
10	10	99.00	100.00	1.00	0.00
20	20	96.00	100.00	4.00	0.00
30	30	91.00	100.00	9.00	0.00
40	40	84.00	100.00	16.00	0.00
50	50	75.00	100.00	25.00	0.00

Example 2. Fluorescence (T Fl) results 5% higher than described:

		% Perennial		% Annual	
% V Fl	% T Fl	Present	Proposed	Present	Proposed
5	10	94.50	95.00	5.50	5.00
10	15	93.50	95.00	6.50	5.00
20	25	90.00	95.00	10.00	5.00
30	35	84.50	95.00	15.50	5.00
40	45	77.00	95.00	23.00	5.00
50	55	67.50	95.00	32.50	5.00

RULES PROPOSAL No. 8

Example a Table II Tolerances for 400 - 1000 seed tests

PRESENT RULE:

Examples —

a. Fluorescence test of ryegrass (chaffy grass)

(1) **Test results:** Pure ryegrass = 98.40%; fluorescence = 9%; nonfluorescence = 81% in a 400-seed test; germination = 90%.

(2) **Calculation of tolerance**

$$\frac{\text{Number of nonfluorescent seeds}}{\text{Number of seeds germinating}} \text{ or } \frac{324}{360} = 90.0\%.$$

Tolerance for fluorescence test result of 90.00 ($\frac{400}{400}$ column) = 3.8 %

1/2 pure seed tolerance (Column D) for 98.40% = $\frac{0.45\%}{\text{Total Tolerance}\quad 4.25\%}$

The tolerance for annual or Italian ryegrass would be figured in a similar manner by making the proper substitutions; thus, the values in

No. (2) above become $\frac{36}{360}$ = 10.00%. The tolerance would be 4.6%

(fluorescence tolerance) plus 0.45% (1/2 pure seed tolerance) equals 5.05%.

(3) **Application of tolerance**

$$\% \text{ perennial ryegrass} = \frac{1.05 \times 81.00 \times 98.40}{90} = 92.99\%$$

% annual or Italian ryegrass = 98.40 - 92.99 = 5.41%

The tolerance is applied to 92.99% or to 5.41% as the case may be.

PROPOSED RULE:

Examples--

a. Fluorescence test of ryegrass (chaffy grass)

(1) Test results: Pure ryegrass = 98.40%; True Fluorescence = 10% in a 400 seed test. Fluorescence level of variety being tested = 0.

(2) Calculation of tolerance

Tolerance for True Fluorescence test result of 10% ($\frac{400}{400}$column) = 4.6%

½ pure seed tolerance (Table 6, Column D) for 98.40% = 0.45%

Total Tolerance 5.05%

(3) Application of tolerance

$\frac{10 - 0}{100}$ x 98.40 = 9.84% annual ryegrass

98.40 - 9.84 = 88.56% perennial ryegrass

The tolerance is applied to 88.56% or 9.84% as the case may be.

SUPPORTING EVIDENCE OR REASONS FOR THE PROPOSED RULE:

Altered to comply with changes proposed in Section 3.5.

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