Rule Change Proposal No. 18

PURPOSE OF PROPOSAL: To include a reference in the Rules for the grow-out test of fluorescent ryegrass seedlings to be used as supplement to fluorescence test of ryegrass. A complete ryegrass grow-out test procedures based on a referee study that was conducted in 2001 will be published in the 'Cultivar Purity Handbook'. This refereed grow-out test procedures will provide standardized protocol to determine the percentage of annual and perennial ryegrass (*Lolium multiflorum* and *L. perenne*) in a ryegrass seed sample. This is a supplemental test to the fluorescent test, not a replacement, to be used primarily in cases when the fluorescence test appears to overestimate annual ryegrass contamination in perennial ryegrass samples (e.g., if test fluorescence level exceeds the VFL of the variety in question, or if requested for verification of fluorescence test results). The new rule will more accurately establish the mechanical purity for reports.

To be included as supplement to section 3.5 'Fluorescence test for ryegrass' in the Rules.

PRESENT RULE: New rule

PROPOSED RULE

Section 3.5 Fluorescence test of ryegrass will be renumbered as 3.5.a.

3.5.b. Procedure for grow-out test of fluorescent ryegrass seedlings to differentiate between annual and perennial types. - Complete protocols for testing, calculation and reporting the grow-out test results for ryegrass are included in the Cultivar Purity Testing Handbook, Contribution No. 33 to the Handbook on Seed Testing, AOSA, 1991, and subsequent updates.

This is an optional, supplemental test to the fluorescence test and is to be used primarily in cases when the fluorescence test appears to overestimate annual ryegrass contamination in a perennial ryegrass sample (e.g., if test fluorescence level exceeds the VFL of the variety in question, or if requested for verification of fluorescence test results). This test is based on "Growth Chamber Testing" procedures described in the AOSA Cultivar Purity Testing Handbook, 1991 that was refereed and revised in 2001. The method is to be used to verify the percentage of annual and perennial growth types of only the fluorescent seedlings in a given ryegrass sample; non-fluorescing seedlings will still be considered an accurate prediction of perennial growth type.

SUPPORTING EVIDENCE

The fluorescent seedling grow-out test (GOT) is proposed as an optional supplement to the ryegrass fluorescent test (FLT) in cases where the fluorescence test appears to overestimate annual ryegrass contamination in perennial ryegrass samples (Oregon Seed Certification Service records indicate FLT significantly overestimates annual contamination in 5-10% of certified seed lots). The GOT is intended to be used only to verify growth type of those seedlings that fluoresce in the FL. Table 1 illustrates the extent of erroneous annual ryegrass determinations that occur in routine testing using the FLT. The FLT overestimates annual ryegrass contamination by over 12% in some samples. Grow-out of the fluorescent seedlings by the GOT clarifies that many fluorescent seedlings have perennial-type growth habit.

Results in Table1, along with published scientific evidence indicate that the fluorescence test is not always an accurate measure of annual ryegrass. Nilsson 1930; Nyquist 1963; Nitzsche 1963; Okora et.al. 1999 showed that both fluorescent perennial and non-fluorescent annual ryegrasses exist. Linehan and Mercer 1933; Woodeforde, 1935; Nyquist 1963; Okora et.al. 1999 reported that none of the annual ryegrass characteristics studied were tightly linked to or associated with the fluorescent trait. In addition, Floyd, 2000 found that environmental conditions of the seed production field affect the level of fluorescence from location to location, and year to year. Therefore, the primary objective of the GOT is to verify annual and perennial ryegrass plant types based on morphological differences among them rather than based solely on the root fluorescence characteristics. Both the AOSA Rules for Testing Seeds (item 3.2a) and the Federal Seed Act Regulations (201.58a) state that "… identification may be based upon the seedling, growing plant or mature plant characteristics…"

Oregon State University Seed Laboratory and USDA-National Forage Seed Production Research Center at Corvallis, OR developed a modified grow-out test protocol. Test procedures were based on those described in the *AOSA Cultivar Purity Testing Handbook, 1991,* but further details were developed and the test adapted for application by seed laboratories. A national referee study was coordinated by the Oregon State University Seed Lab in 2001 to (1) standardize test procedures, and (2) measure the variability in results among the participating labs. A total of 11 laboratories participated in the study.

Each participating lab received four samples. Two samples were from randomly selected commercial seed lots: sample'L-1' had VFL = 5.00% and sample 'BR-2' had VFL = 1.79% (VFL is the Variety Fluorescence Level as described by the breeder of each variety indicating the expected percentage of perennial types that will fluoresce). The other two samples, 'CH-5' and 'CH-10', were 'made up' samples consisting of 95 and 90% of a perennial ryegrass cultivar (VFL of 0.54%), and artificially contaminated with 5 and 10% of Gulf annual ryegrass (VFL 99.02%), respectively. The percentage of pure seeds in each of the four samples was recorded and used to determine the percentage of annual-type using the fluorescence test formulas described in the AOSA 'Rules for Testing Seeds', 1998, page 16. In our discussion, we will use 'FLT' to refer to the percentage of annual ryegrass found in a perennial sample that was calculated based on the fluorescence test results.

All laboratories were asked to follow the same protocol as closely as possible using standardized conditions of light, temperature, water, nutrients, sample size, criteria for classification of annual and perennial types, and the duration required to complete the test.

Results from two of the 11 participating labs were not included in the final statistical analysis because protocol or seedling classification deviances rendered their results outside of normal variation. In one case erroneous classification of annual and perennial types created an additional identification category that could not be compared with results from the other participating labs. Another lab terminated the test too early and reported only 2.3% annual types in the sample that had 5% known annual type, and only 6% in the sample that had 10% known annual type.

The experimental model used in this study was a one factor (labs) randomized complete block design with four replications of 100 seeds each. Each of the four samples was analyzed separately since comparison between samples was not one of the objectives of this study.

The analysis of variance results of the grow-out test showed almost no significant differences among the nine labs participating in the study (Table 2). These results suggest that by closely following the test protocol, laboratories produced consistent results for a GOT. Tables 3-6 list annual ryegrass contamination values for all four samples by all nine labs as determined by both the fluorescence test (FLT) and the grow-out test (GOT). Close agreement between the two test methods is confirmed in comparing percentage annual types detected, and in noting the low variance among labs. For comparison, Table 2 shows that germination test variance among the labs was highly significant.

Unfortunately, none of the samples used in this referee illustrated the situation where FLT significantly overestimates annual ryegrass contamination, so the referee test demonstrated that both FLT and GOT estimate the same contamination in these cases. Sample 'BR-2' does show an average 2.7% annual type content by FLT and only 0.6% by GOT (Table 4).

The objective of including the two known, pre-determined annual/perennial mixtures samples, CH-5 and CH-10, was to determine if all participating labs, using the same protocol, would consistently detect the known number of annual seeds that were deliberately included in the samples. Doing so reduced sampling variability, and focused on the variation due to labs and protocol parameters. Using the grow out test, six out of the nine labs detected at least 4.5% annual ryegrass contamination in CH-5 while three labs detected 4.0-4.5% (Table 5). Seven out of the nine labs detected 8.9-10% contamination in CH-10, which included 10% annual ryegrass seeds; one lab detected 8% and the lowest detected 7% (Table 6). Variances in detecting known annual contaminants were very similar for the fluorescence and grow out test methods (Tables 3-6).

Personal communication with the labs participating in the study indicated that analysts with limited experience in conducting the GOT achieved less accurate results, which may have contributed to the variation in results among labs. Therefore, knowledge of differentiation between annual and perennial types based on morphological differences such as heading, stem elongation, blade width and color is a key for objective, accurate evaluation and results.

Three out of the nine labs finished the test in 35 days achieving results as accurate as other labs that finished the test in 41 or 42 days. This indicates that the test can be completed in 35 days providing that light intensity and other recommended test parameters (i.e., temperature, water nutrients, media) are met, and the analysts have reasonable experience in test evaluation. Proper judgment of test completion is ensured by observing full heading of the annual ryegrass check plants as prescribed in the test protocol.

Two out of the nine labs used greenhouses instead of growth chambers. Although both greenhouses attempted to regulate temperature at constant 25°C, actual temperatures ranged between 15-31°C. Yet, these labs had consistent results comparable with those using growth chambers with constant 25°C ± 2°C. Moreover, one of the labs using a greenhouse successfully completed the test in 35 days, suggesting that neither the minimum nor the maximum temperatures in the greenhouses lasted long enough to significantly affect plant growth and heading. It is possible that alternating temperatures may speed up heading in case of the lab that completed the test in 35 days.

Natural light (in greenhouse), which has a wider spectrum than artificial light sources (i.e. cool white florescent tubes and incandescent bulbs) may accelerate growth and speed up flowering of plants. The results of this study suggest the use of continuous light (Nittler and Kenny, 1972) with a minimum light intensity of 1200 foot candle (232 µmol m^2 s^{-1}) during the day, and 800 f.c. during the night produce satisfactory results. Lower light intensity may result in extending the time needed to complete the test, whereas higher light intensity may speed up the heading process.

As with any biological test, some variation among test results was expected due to variability in genetic and physiological condition of different seeds within the same sample; subjectivity in evaluation; and/or deviation from the recommended test conditions. However, observed variances in the grow-out tests within and among labs were significantly low. For comparison, although all labs followed the same recommended AOSA standard germination test procedures, variation in germination results was much higher than for either the GOT or the FLT (Tables 2-6; Figures 1-4).

Table 1. Percentage of Annual Ryegrass seeds in 45 Perennial Ryegrass samples tested at the Oregon State University Seed Laboratory using Fluorescence and Grow-out Tests.

Lot No.	*Variety*	% Annual Type Based on		Differ-ence	Lot No.	Variety	% Annual Type Based on		Differ-ence
		Fluor-escence Test	Grow out Test				Fluor-escence Test	Grow out Test	
1	Accent	2.61	0.26	2.35	23	Imagine	13.0	2.36	10.64
2	Caddieshack	4.29	0.0	4.29	24	Imagine	18.38	4.13	4.25
3	Continental	14.55	6.78	7.77	25	Imagine	6.63	1.09	5.54
4	Continental	10.44	3.23	7.21	26	Imagine	5.32	0.0	5.32
5	Continental	8.42	2.75	5.67	27	Imagine	10.51	1.61	8.90
6	Continental	13.51	4.97	8.54	28	Imagine	11.26	3.36	7.9
7	Continental	11.7	5.48	6.22	29	Imagine	9.59	1.4	8.19
8	Continental	16.31	8.42	7.89	30	Imagine	15.7	3.27	12.43
9	Continental	15.82	7.17	8.65	31	Imagine	4.05	0.27	3.78
10	Continental	16.76	6.24	10.52	32	Monterey II	2.10	0.0	2.10
11	Continental	15.01	4.78	10.23	33	Peak	6.17	3.21	2.96
12	Continental	17.24	6.81	10.43	34	Pinnacle	3.42	0.78	2.64
13	Continental	8.88	3.85	5.03	35	Pinnacle	13.8	7.23	6.57
14	Continental	12.73	4.97	7.76	36	Pinnacle	2.34	1.17	1.17
15	Continental	15.12	6.12	9.00	37	Prelude III	6.49	0.77	5.72
16	Derby Supreme	8.95	0.0	8.95	38	Prelude III	7.29	0.0	7.29
17	Derby Supreme	7.14	0.0	7.14	39	Prelude III	7.95	1.0	6.95
18	Derby Supreme	6.99	1.01	5.98	40	Prelude III	8.97	1.04	7.93
19	Derby Supreme	11.11	1.28	9.83	41	R2	8.03	3.82	4.21
20	Derby Supreme	6.53	0.51	6.02	42	R2	3.47	0.53	2.94
21	Derby Supreme	6.82	1.04	5.78	43	Regal	20.92	9.20	11.72
22	Derby Supreme	6.67	0.77	5.90	44	Regal	15.18	6.49	8.69
					45	Regal	19.05	9.99	9.06
Grand Mean							*10.07*	*3.11*	*6.96*

Table 2. Analysis of variance of germination and percentage of annual type in four perennial ryegrass samples as determined by fluorescence test (FLT) and grow-out test (GOT) at 9 laboratories.

Source	df	*Germination*				*% Annual type*							
						FLT				*GOT*			
		CH-5	CH-10	L-1	BR-2	CH-5	CH-10	L-1	BR-2	CH-5	CH-10	L-1	BR-2
Replication	3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Labs	8	***	***	*	ns	ns	ns	*	ns	ns	ns	ns	ns

* Indicates significance at P≤0.05, *** indicates significance at P≤ 0.001, and ns indicates not significance at P≤0.05.

Table 3. Variation among 9 seed laboratories in determining the percentage of annual and perennial ryegrass of sample L-1 using the fluorescence test (FLT) and the grow-out test (GOT).

Labs	*% Germination*	*% Annual type*	
		FLT	*GOT*
1	93	5.3	5.7
2	94	.7	6.8
3	89	6.3	5.7
4	90	6.1	7.2
5	91	7.2	5.4
6	88	8.8	6.1
7	91	3.9	4.0
8	90	5.3	4.7
9	92	6.8	6.3
Mean	***91***	***6.0***	***5.8***
LSD (0.05)	***3.0***	***2.9***	***2.9***

Table 4. Variation among 9 seed laboratories in determining the percentage of annual and perennial ryegrass of sample BR-2 using the fluorescence test (FLT) and the grow-out test (GOT).

Labs	*% Germination*	*% Annual type*	
		FLT	*GOT*
1	96	2.4	0.8
2	94	2.7	0.8
3	97	2.6	0.8
4	96	2.2	0.3
5	96	4.2	0.6
6	91	3.2	0.6
7	95	2.0	0.0
8	95	2.2	0.3
9	96	3.2	1.0
Mean	***95***	***2.7***	***0.6***
LSD (0.05)	***3.0***	***2.8***	***1.3***

Table 5. Variation among 9 seed laboratories in determining the percentage of annual and perennial ryegrass of sample CH-5 using the fluorescence test (FLT) and the grow-out test (GOT).

Labs	*% Germination*	*% Annual type*	
		FLT	*GOT*
1	99	4.2	4.8
2	98	*4.3*	4.8
3	94	4.6	5.3
4	97	4.3	4.6
5	98	4.8	4.0
6	96	4.6	4.1
7	98	4.0	4.5
8	97	4.1	4.1
9	98	4.3	4.8
Mean	***97***	***4.4***	***4.6***
LSD (0.05)	***2.0***	***1.4***	***1.3***

Table 6. Variation among 9 seed laboratories in determining the percentage of annual and perennial ryegrass of sample CH-10 using the fluorescence test (FLT) and the grow-out test (GOT).

Labs	*% Germination*	*% Annual type*	
		FLT	*GOT*
1	99	9.3	9.8
2	97	7.7	7.9
3	90	8.9	9.4
4	98	8.4	8.9
5	97	10.5	6.8
6	96	10.1	8.9
7	96	9.5	10.0
8	99	8.7	9.0
9	97	9.7	10.2
Mean	***97***	***9.2***	***9.0***
LSD (0.05)	***2.2***	***1.8***	***2.1***

Conclusion:

Significant uniformity in GOT results was found among the nine labs used in the study when they followed the same protocol. These results clearly support the proposed GOT protocol as a valid, reliable supplement to the fluorescence test for verifying annual and perennial ryegrass types when the fluorescence test over estimates annual ryegrass contamination in a perennial ryegrass sample. Although differences in light intensity can affect the length of time to achieve heading, the GOT can be completed within 35-42 days after transplanting by following the recommended continuous light regime.

References:

AOSA. 1991. Cultivar Purity Testing Handbook. Association of Official Seed Analysts, Lincoln, NE

AOSA. 2000. Rules for Testing Seeds. Association of Official Seed Analysts, Lincoln, NE.

Floyd, DJ (2000) Ryegrass Fluorescence expression during three generations of seed increase and its heritability among half-sib families. Ph.D. thesis, Oregon State University.

Linehan, PA and SP Mercer (1933). Fluorescence of *Lolium* seedlings in ultraviolet light. Nature (London) 131: 202-203.

Nilsson, F (1930). Einige resulte vonn isolations- und bastardierrung versuchen mit *Lolium multiflorum* Lam. und *Lolium perenne* L. Bostaniska notiser 3-4:161-166.

Nittler, LW and TJ Kenny (1972). Distinguishing annual from perennial ryegrass. Agron. J. 64:767-768.

Nitzsche, VW (1963). Nichtfluoreszierendes welsches weidelgras (*Lolium multiflorum* Lam). Der Zucher 33:281-282.

Nyquist, WE (1963). Fluorescent perennial ryegrass. Crop Sci. 3:223-226.

Okra, JO, Watson, CE, Gourley, LM, Keith, BC and CE Vaughn (1999). Comparison of botanical characters and seedling root fluorescence for distinguishing Italian and perennial ryegrass. Seed Sci. & Technol. 27:721-730.

Woodforde, AH (1935). The inheritance of a substance in the roots of seedling hybrid derivatives of *Lolium perenne* L. x *Lolium multiflorum* Lam., causing a fluorescence reaction visible in filter paper by screened ultra-violet light. J. Linnean Soc. London 50:141-150.

Grow-Out Protocol of Fluorescent Ryegrass Seedlings To Differentiate Between Annual And Perennial Types Based on a Referee Study as it Should Appear in the AOSA Cultivar Purity Handbook:

***Germination and fluorescence test*:** Germinate 400 seeds following the AOSA Rules, section 4.8i, and then determine the number of seedlings that fluoresce under ultra-violet light at the end of the germination test period.

Transplanting and sample size: Transplant all fluorescent seedlings, along with a minimum of 20 random non-fluorescing perennial ryegrass seedlings from the sample being tested (perennial ryegrass check), and a minimum of 20 annual ryegrass seedlings (annual ryegrass check). To ensure test uniformity among labs, a typical 'Gulf' variety seed lot will be available from Oregon State University Seed Laboratory for all US labs to use as an annual ryegrass check.

Planting substrate and containers: A high quality peat based potting soil is recommended to assure high water holding capacity. Pots, flats, or cell planting trays (minimum 5 cm diameter and 7 cm depth) should have perforated bottoms to drain excess water. Planting substrate should be moistened prior to transplanting to provide enough moisture for the roots of transplanted seedlings. Seedlings should be spaced at least 5 cm apart for easier evaluation.

***Water and nutrient requirement*:** Transplants should be watered as needed and fertilized weekly with Hoagland's No. 1 nutrient solution (as described in the AOSA Cultivar Purity Testing Handbook, 1991) or by another commercial fertilizer that contains macro- and micronutrients.

***Temperature*:** Maintain 25°C temperature in growth chambers or greenhouses. Although greenhouse temperatures may fluctuate within a range of 15°C, the minimum and maximum temperatures usually should not last long enough to affect the growing plants significantly.

***Light*:** Provide continuous light, minimum 1200 f.c. or 232 μmol $m^{-2}s^{-1}$ PAR using high pressure sodium lamps, or cool white fluorescent tubes and incandescent bulbs. In general, increasing light intensity, increases speed of flowering and heading. Light intensity in the greenhouse may drop to less than 1200 f.c. during cloudy days, but does not significantly reduce growth and heading rate.

Growth chamber, greenhouse or walk-in germination room can be used, provided that the light and temperature requirements and the other test conditions described above are met.

Test period and final evaluation: Plants should be evaluated when at least 75% heading of the annual ryegrass check sample is achieved (usually about 35-42 days after transplanting).

***Plants should be classified as follows*:**

a) *Perennial type:* Plants that have not headed and have characteristics similar to the non-fluorescing perennial ryegrass check plants.

b) *Annual type*: Plants that have headed, do not resemble the perennial ryegrass checks, and/or resemble the annual ryegrass checks more closely than the perennial ryegrass checks (e.g., have wider blades, lighter color, and elongated stems).

Calculations:

$$\%\,Annual\ \ ryegrass = \frac{Number\ of\ \ fluorescent\ \ plants\ that\ have\ headed\ or\ resemble\ annual\ checks}{Total\ number\ of\ \ normal\ \ seedlings - mortality\ \ factor}\ X\ \%Pure\ ryegrass$$

$$\%\ Perennial\ ryegrass = \%\ Pure\ ryegrass - \%\ Annual\ ryegrass$$

Example 1. *(no mortality during the grow-out test)*

% of pure ryegrass (obtained in the physical purity analysis) = 99.12
Normal seedlings (obtained in the germination test out of 400 seeds planted)= 380
Number of normal fluorescent seedlings to be grown out = 12
Number of plants that died during or after transplanting = 0
Number of annual-type plants at the end of the grow-out test = 4

$$\% \text{ Annual ryegrass} = \frac{4}{380 - 0} \; x \; 99.12 \; = \; 1.04$$

% Perennial Ryegrass = 99.12 – 1.04 = 98.08

***Mortality calculation*:**
If any fluorescent seedling dies during transplanting and grow-out, before the final evaluation is completed, a proportional adjustment must be made in the total number of normal seedlings to account for this mortality (if mortality exceeds 25% of the total transplanted fluorescent seedlings, the test must be repeated). The following formula should be used:

$$\text{Mortality factor} = \frac{\text{No. of seedlings died during transplanting and growout}}{\text{Total No. of fluorescent seedlings}} \; x \; \text{Total No. of normal seedlings}$$

Example 2. *(In case of mortality during the grow-out test)*

% of pure ryegrass (obtained in the purity analysis) = 99.12
No. of normal seedlings (out of 400 seeds planted) = 380
No. of fluorescent seedlings = 12
No. of plants that died during or after transplanting = 3
Number of annual type plants at the end of the grow-out test = 4

$$\text{Mortality factor} = \frac{3 \quad x \quad 380}{12} \quad = \quad 95$$

$$\% \text{ Annual ryegrass} = \frac{4}{380 - 95} \; x \; 99.12 \; = \; 1.39$$

% Perennial Ryegrass = 99.12 – 1.39 = 97.73

Reporting test results: Purity results based on the grow-out test may differ from those based on the fluorescence test in both the 'pure seed' and 'other crop' components. When the grow-out test is used to determine the percentage of annual and perennial ryegrass in a sample, the purity report should include the following statement: "Pure seed is based on grow-out test". A grow-out test report would include the percentages of perennial and annual types.

***Tolerances for grow-out tests*:** Table 4 in Sec. 201.62 of the FSA Regulations (it is also included in the AOSA Rules for Testing Seeds as Table 10) contains tolerances appropriate for use when comparing test results of grow-out tests (copy following). In Table 4, find the maximum allowable tolerance by using the number of normal seeds evaluated in the fluorescence test at the top row (No. of seeds, seedlings or plants in the test; usually 400); and the percentage of perennial or annual type as calculated by the grow-out test formulas at the side column (Seed, seedling or plant count %).

Table 4. Tolerances for Purity Tests, When Results Are Based on 10 to 1,000 Seeds, Seedlings, or Plants Used in a Test (from FSA Regulations 201.62)

Seed, seedling or Plant count percent	Number of seeds, seedlings, or plants in tests										
	10	20	30	50	75	100	150	200	400	800	1000
100 or 0	0	0	0	0	0	0	0	0	0	0	0
98 or 2	10.3	7.3	6.0	4.6	3.8	3.3	2.7	2.3	1.6	1.2	1.0
96 or 4	14.4	10.2	8.3	6.4	5.3	4.6	3.7	3.2	2.3	1.7	1.5
94 or 6	17.5	12.4	10.1	7.8	6.4	5.5	4.5	3.9	2.9	2.1	1.9
92 or 8	20.0	14.1	11.5	8.9	7.3	6.3	5.2	4.5	3.4	2.4	2.2
90 or 10	22.1	15.7	12.8	9.9	8.1	7.0	5.7	4.9	3.8	2.8	2.4
88 or 12	24.0	17.0	13.8	10.7	8.7	7.6	6.2	5.4	4.1	3.0	2.7
86 or 14	25.7	18.1	14.7	11.4	9.3	8.1	6.6	5.7	4.5	3.2	2.9
84 or 16	26.9	19.0	15.5	12.1	9.8	8.5	7.0	6.0	4.8	3.4	3.0
82 or 18	28.2	20.0	16.4	12.6	10.3	8.9	7.3	6.3	5.0	3.6	3.2
80 or 20	29.5	20.9	16.9	13.2	10.7	9.3	7.6	6.6	5.3	3.8	3.3
78 or 22	30.5	21.6	17.6	13.6	11.0	9.6	7.9	6.8	5.5	3.9	3.5
76 or 24	31.4	22.3	18.2	14.1	11.5	9.9	8.1	7.0	5.7	4.1	3.6
74 or 26	32.3	22.8	18.6	14.4	11.8	10.2	8.3	7.2	5.8	4.2	3.7
72 or 28	33.0	23.4	19.0	14.8	12.1	10.5	8.5	7.4	6.0	4.3	3.8
70 or 30	33.7	23.8	19.5	15.1	12.3	10.7	8.7	7.5	6.2	4.4	3.9
68 or 32	34.3	24.3	19.9	15.4	12.5	10.8	8.9	7.7	6.3	4.5	4.0
66 or 34	35.0	24.7	20.2	15.7	12.7	11.0	9.0	7.8	6.4	4.6	4.0
64 or 36	35.4	25.0	20.5	15.8	12.9	11.2	9.1	7.9	6.5	4.6	4.1
62 or 38	35.5	25.4	20.6	15.9	13.0	11.3	9.2	8.0	6.6	4.7	4.2
60 or 40	36.1	25.7	20.9	16.1	13.2	11.4	9.3	8.1	6.7	4.8	4.2
58 or 42	36.2	25.7	21.0	16.2	13.3	11.5	9.4	8.1	6.8	4.8	4.2
56 or 44	36.5	25.8	21.0	16.4	13.3	11.5	9.4	8.2	6.8	4.8	4.3
54 or 46	36.8	25.8	21.2	16.4	13.4	11.6	9.5	8.2	6.9	4.9	4.3
52 or 48	36.8	25.9	21.2	16.5	13.4	11.6	9.5	8.2	6.9	4.9	4.3
50	36.8	25.9	21.3	16.5	13.4	11.6	9.5	8.2	6.9	4.9	4.3

Example 3:

An initial grow-out test reported 98.0% perennial ryegrass, but a subsequent test showed only 94.4%. In the second test 380 normal seedlings were obtained out of the 400 seeds planted for the fluorescence test. Are these two samples within tolerance?

Apparent test discrepancy = 98 – 94.4 = 3.6%
Average of the two tests is (98 + 94.4)/2 = 96.2, rounded to 96%
In Table 4, 380 is between column 200 and 400 at row '96 or 4', so the tolerance is between 3.2 and 2.3 and must be computed by interpolation. Since 380 plants is 180/200 of the way between 200 and 400, then the allowable tolerance is 180/200 of the way between 3.2 to 2.3.
Therefore, the interpolated tolerance is: 3.2 - [(3.2 – 2.3) x 180/200)] = 2.4.
The test discrepancy of 3.6 exceeds the tolerance 2.4, so the tests are out of tolerance.

SUBMITTED BY: Adriel Garay and Sabry Elias
Oregon State University Seed Laboratory
Phone: (541) 737-4499 Fax: (541) 737-2126
e-mail: adriel.garay@orst.edu or sabry.elias@orst.edu

DATE SUBMITTED: October 15, 2001 **REVISED**: December 21, 2001