

2018 Rule Proposal 9

Purpose of Proposal

Add an alternative method to evaluate the Grow-out test of fluorescent ryegrass seedlings in the Rules vol. 1, sec. **5.2.c. Grow-out test of fluorescent ryegrass seedlings**: To verify annual and perennial growth habits of fluorescent ryegrass seedlings. **Also, add the same alternative method** in the protocol of grow-out test of fluorescent ryegrass seedlings **in the AOSA Cultivar Purity Testing Handbook, 2008** and subsequent updates.

Present Rule

In the AOSA Rules vol. 1

5.2. c. Grow-out tests

Grow-out test of fluorescent ryegrass seedlings: To verify annual and perennial growth habits of fluorescent ryegrass seedlings.

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 (i.e., when the test fluorescence level exceeds the VFI of the variety in question, or when verification of fluorescence test results is requested).

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.

Complete protocols for testing, calculation and reporting the grow-out test results for ryegrass are included in the AOSA Cultivar Purity Testing Handbook, Contribution No. 33 to the Handbook on Seed Testing, AOSA, 2008, and subsequent updates.

In the AOSA Cultivar Purity Testing Handbook, 2008

Grow-Out of Fluorescent Ryegrass Seedlings to Differentiate Between Annual and Perennial Types

Procedure

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

Transplanting and sample size: From the sample being tested, transplant all fluorescent seedlings and at least 20 random non-fluorescing seedlings (perennial ryegrass check). In addition, plant at least 20 annual ryegrass seedlings (annual ryegrass check) from a known annual ryegrass source. To ensure test uniformity among labs, a typical ‘Gulf’ variety seed lot will be available from Oregon State University Seed Laboratory for all US laboratories to use as an annual ryegrass check.

Planting substrate and containers: A high quality peat based potting soil is recommended to assure adequate plant support and high water holding capacity. Use of Pots, flats, or cell planting trays (minimum 5 cm diameter and 7 cm depth) with perforated bottoms is recommended to drain excess water. Planting substrate should be moistened prior to transplanting to provide adequate 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 previously described in this Handbook) or by other fertilizers that contains macro- and micronutrients.

Temperature: Maintain 25°C in growth chambers or greenhouses. Although greenhouse day and night temperatures may fluctuate within a range of approximately ±8°C, the minimum and maximum temperatures usually do not last long enough to affect the growing plants significantly.

Light: Provide continuous light with a minimum of 1200 foot candles or 232 μmol m⁻²s⁻¹ using high pressure sodium lamps, or a combination of cool white fluorescent tubes and incandescent bulbs. In general, increasing light intensity speeds up flowering and heading. Light intensity in the greenhouse may drop to less than 1200 foot candles during cloudy days, but it does not appear to reduce growth and heading rate. A growth chamber, greenhouse or walk-in germination room can be used, provided that the test conditions described above are met.

Test period and final evaluation: Plants should be evaluated when at least 80% of the annual ryegrass check sample has headed (usually about 35-42 days after transplanting).

Classification: Plants should be classified as follows:

- a) *Perennial type:* Plants that have not headed and have characteristics similar to the nonfluorescing 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 and intermediate/off types, e.g., have wider leaf blades, lighter color, and elongated stems.

Formulas and calculations:

$$\% \text{Annual ryegrass} = \frac{\text{Total number of normal seedlings} \times \text{Survival factor}}{\text{Number of fluorescent plants that have headed or resemble annual checks}} \times \text{Pure ryegrass}$$

$$\% \text{Perennial ryegrass} = \% \text{Pure ryegrass} - \% \text{Annual ryegrass}$$

Survival factor formula: If any fluorescent seedling dies during transplanting and/or the growout period, before the final evaluation is completed, a proportional adjustment must be made to account for this mortality. Only survived plants should be considered in the calculation. If mortality exceeds 25% of the total transplanted fluorescent seedlings, the test must be repeated. The following formula should be used:

$$\text{Survival factor} = \frac{\text{No of fluorescent seedlings} - \text{No of fluorescent seedlings that died during the grow out test}}{\text{No of fluorescent seedlings}}$$

Example 1

% 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

$$\text{Survival factor} = (12 - 3)/12 = 0.75$$

$$\% \text{Annual ryegrass} = 4 / (380 \times 0.75) \times 99.12 = 1.39$$

$$\% \text{Perennial Ryegrass} = 99.12 - 1.39 = 97.73$$

Tolerances for grow-out tests: The enclosed tolerances in Table 2.3 are adopted from Table V1 ‘Trueness to variety’, two estimates, one-way test at 5% significance level, Handbook of Tolerances, Miles 1963, which is the same as Table 4 in Sec. 201.62 of the FSA Regulations. It is also included in the AOSA Rules for Testing Seeds as Table 10.

Proposed Rule

In the AOSA Rules vol. 1

5.2. c. Grow-out tests

Grow-out test of fluorescent ryegrass seedlings: To verify annual and perennial growth habits of fluorescent ryegrass seedlings.

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 (i.e., when the test fluorescence level exceeds the VFL of the variety in question, or when verification of fluorescence test results is requested).

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. **Two alternative methods can be used to determine test period and final classification of plants into annual and perennial types.**

Complete protocols for testing, **methods of evaluation**, calculation and reporting the grow-out test results for ryegrass are included in the AOSA Cultivar Purity Testing Handbook, Contribution No. 33 to the Handbook on Seed Testing, AOSA, 2008, and subsequent updates.

In the AOSA Cultivar Purity Testing Handbook, 2008

Grow-Out of Fluorescent Ryegrass Seedlings to Differentiate Between Annual and Perennial Types

Two alternative methods can be used to determine test period and final classification of plants into annual and perennial types:

I. Based on plant heading

Test period and final evaluation: Plants should be evaluated when at least 80% of the annual ryegrass check sample has headed (usually about 35-42 days after transplanting).

Classification Criteria: 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 and intermediate/off types, e.g., have wider leaf blades, lighter color, and elongated stems.

This type of evaluation is appropriate for the forage perennial type with high VFL, Italian ryegrass (i.e., biennial, which behave first year like perennial and needs vernalization to head), intermediate ryegrass, and upon customer request.

II. Based on leaf morphology

Test period and final evaluation: Depending on the way of planting seeds directly or transplanting seedlings after the fluorescent test, final evaluation should be conducted after two test periods:

1. 21 days after transplanting fluorescent seedlings into the greenhouse.
2. 28 days after direct planting of ryegrass seeds in the greenhouse (without fluorescent test).

Classification Criteria: Plants should be classified as follows:

- a) *Perennial type:* if leaves have characteristics similar to the non-fluorescing perennial ryegrass check plants (e.g., narrow leaf blades, darker green, and shorter stems compared to the annual control type).
- b) *Annual type:* If leaves have characteristics that do not resemble the perennial ryegrass checks, and/or resemble the annual ryegrass checks more closely than the perennial ryegrass checks and intermediate/off types, e.g., have wider leaf blades, lighter color, and elongated stems.

This type of evaluation is appropriate for the turf perennial type with VFL below 40%

Supporting evidence (See the attached file for details)

The current grow-out test (GOT) takes long time, up to eight weeks to complete, two of which in germination/fluorescence test and 5-6 weeks for the grow out test in the greenhouse (GH). The grow-out test would remain as realistic test that differentiate between annual and perennial types based on the morphological appearance of the plants. However, shorter testing time, while keeping the accuracy of results would save time and money.

Three studies have been conducted to explore a shorter method to distinguish ARG from PRG using leaf morphology instead of the current forming heads criteria

- I. Study 1. Measure the precision** of evaluating the GOT based on Leaf morphology in **spiked samples**.
- II. Study 2. Measure the effectiveness of** evaluating the GOT based on Leaf morphology in **turf type of PRG with low VFL**.
- III. Study 3. Measure the effectiveness of** evaluating the GOT based on Leaf morphology in **high VFL, forage type of PRG**.

Summary of results (See the attached file for details)

Study 1

- Plant height, leaf width and color provided reliable indicators for separating ARG and PRG type regardless of the level of the contamination of PRG samples with annual type.
- Final evaluation of GOT can be made reliably after 3 or 4 weeks of transplanting, shortening the testing time by 2-3 weeks compared to evaluation based on the plant heading criterion.

Study 2

- Plant height, leaf width and color were found to be reliable indicators for separating turf type PRG from ARG at 3rd - 4th week after transplanting.
- Two-three weeks can be saved using the leaf morphology criterion, i.e., plant height, leaf width and color compared to waiting for the plants to form heads.

Study 3

- Morphological characteristics of plant height, leaf width, and color are not reliable traits to distinguish between high VFL or forage type of PRG and annual ryegrass.
- Waiting for the plants to form heads must be used to differentiate between high VFL or forage types of PRG and ARG.

Overall Conclusions

- Plant height, leaf width and color were found to be reliable indicators for differentiation between turf type PRG and ARG in the grow-out test (GOT).
- The test period for reliable evaluation of GOT based on leaf morphology and the separation between PRG turf and ARG types is 3 - 4 weeks. This would save 2-3 weeks of test period compared to waiting for the plants to head, if heading criterion is used.
- Plant heading trait should remain as the only criterion for separating high VFL and forage PRG types from ARG.

Harmonization

ISTA does not have grow-out test to differentiate annual and perennial types in its Rule. FSA has a grow-out test based on the heading plants of annual as a criterion to distinguish between the two types.

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Date Submitted: October 15, 2017