

The Value of Fluorescence and Grow-out Tests in Differentiating Annual and Perennial Ryegrass

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Both the fluorescence (FL) and the grow-out (GO) tests have been used to differentiate between annual ryegrass (ARG) and perennial ryegrass (PRG) for many years. The grow-out is an optional, supplemental test to the fluorescence test, and is used when the fluorescence test appears to overestimate annual contamination in perennial lots (those lots that exceed 3% over their respective VFL values), or to verify the growth habits of fluorescent ryegrass seedlings.

Fluorescence Test

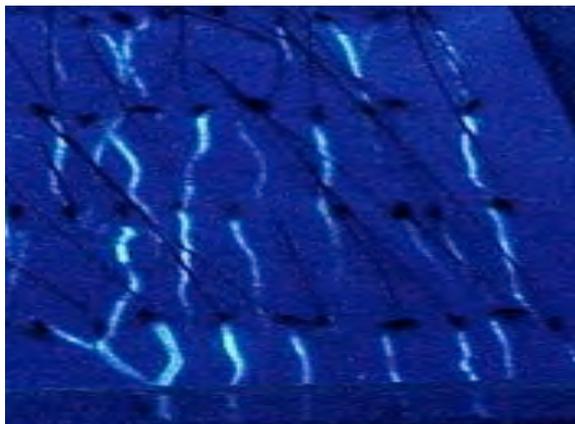


Figure 1. At the end of the germination test, fluorescent ryegrass roots are determined to indicate annual type.

Advantages

- Simple and cheap.
- As fast as the germination test.
- Accepted by AASCO, AOSA and AOSCA.

Disadvantages

- Fluorescence trait is not always associated with annual type.
- In some cases, it overestimates annual contamination in perennial lots.

Grow-out Test

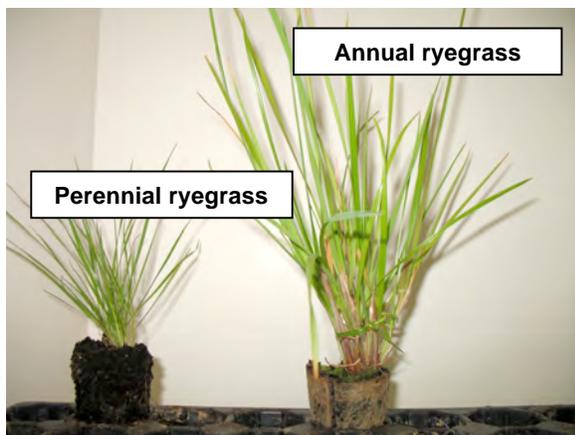


Figure 2. Annual ryegrass has wider blades, lighter color and longer stems than perennial type.

Advantages

- Gives realistic results because it is based on the morphological appearance of the plants.
- Accepted by AASCO, AOSA and AOSCA.

Disadvantages

- It takes 5-6 weeks after the germination test.

Since Rampton (1938) found that 94% of the annual seed lots he screened in Oregon fluoresced, no studies were conducted to measure the bias of FL and GO tests in distinguishing between annual and perennial types. Therefore, a study was conducted in 2010 at OSU Seed Lab to validate this report.

The Value of the Fluorescence Test

- The OSU study used samples from over 7,000 Oregon perennial ryegrass lots from 2007-2009 representing 224 cultivars.
- The study showed that 94% of all perennial ryegrass samples across three years met Oregon certification standard (i.e., were within 3% of their respective VFL values).
- Only 6% out of all samples that were tested over the three-year study period exceeded the 3% tolerance over their respective VFL values (Fig. 3). The grow-out test can be used in such cases to determine if this is due to overestimation or true contamination (Fig. 4).
- The results showed that the fluorescence test is suitable to distinguish between annual and perennial ryegrass in most cases (94%).

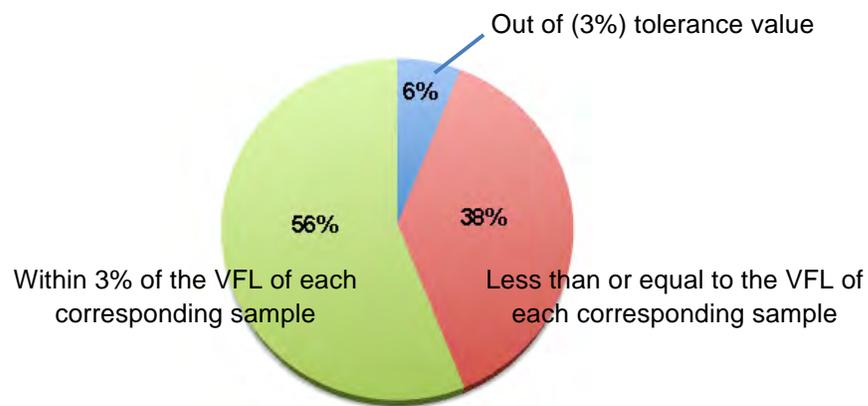


Figure 3. Average of fluorescence test results of 7,703 PRG samples tested over the 3-year period.

The Value of the Grow-out Test

- 90% of the samples that were tested by the grow-out over the 3-year period were within 3% of their respective VFL values.
- Figure 4 shows various examples for the usefulness of the grow-out test in providing realistic information about the presence of annual type in perennial seed lots.
- The grow-out test gives realistic assessment of ryegrass types based on morphological appearance and growth habits of plants. It detects the magnitude of real contamination regardless of the fluorescence level.
- The grow-out test indicates the phenotypic expression of the genetic make up of seeds; therefore, it is a good point of reference for DNA tests.

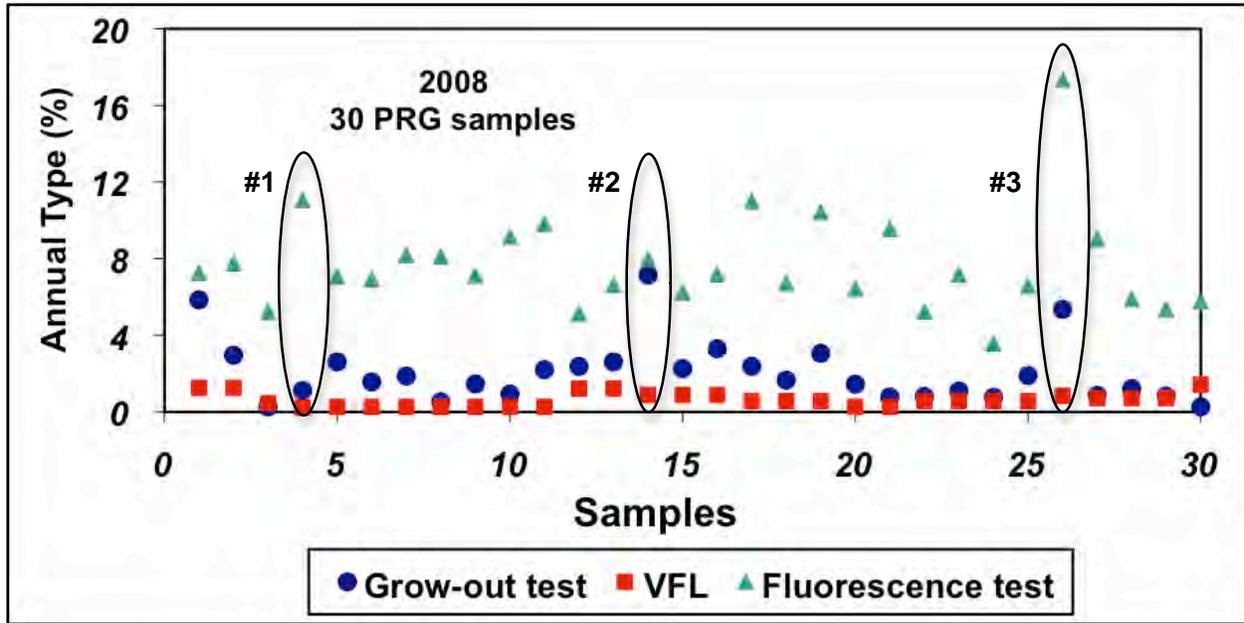


Figure 4. Comparison among FL test, GO test, and VFL of 30 PRG samples in 2008. Sample #1 is an example when the FL test overestimates the annual type, but the GO result was similar to the VFL. Sample #2 is an example when the GO test confirmed the FL test result. Sample #3 FL test overestimates annual type, but the GO test determined the actual contamination compared to the VFL value of that lot.

How is a grow-out test conducted? Its application and benefits

At the end of a fluorescence test, all fluorescing seedlings of the sample being tested, along with annual and perennial control check samples, are planted side by side in a controlled greenhouse environment (Fig. 5). The transplanted seedlings grow for approximately 35-42 days.



Figure 5. Grow-out test of ryegrass at the greenhouse of Oregon State University Seed Lab. Cell-planting trays are used to transplant fluorescent seedlings for accurate evaluation.

At the end of the period, the plants are classified into annual type or perennial type (Fig. 6) according to the AOSA Cultivar Purity Testing Handbook.

When the purity report is based on this test, the laboratories are required to state “annual/perennial determination is based on grow-out test”. Given that this test shows the phenotype of plants, this test can be used as a reference for future DNA tests that try to determine various plant features.

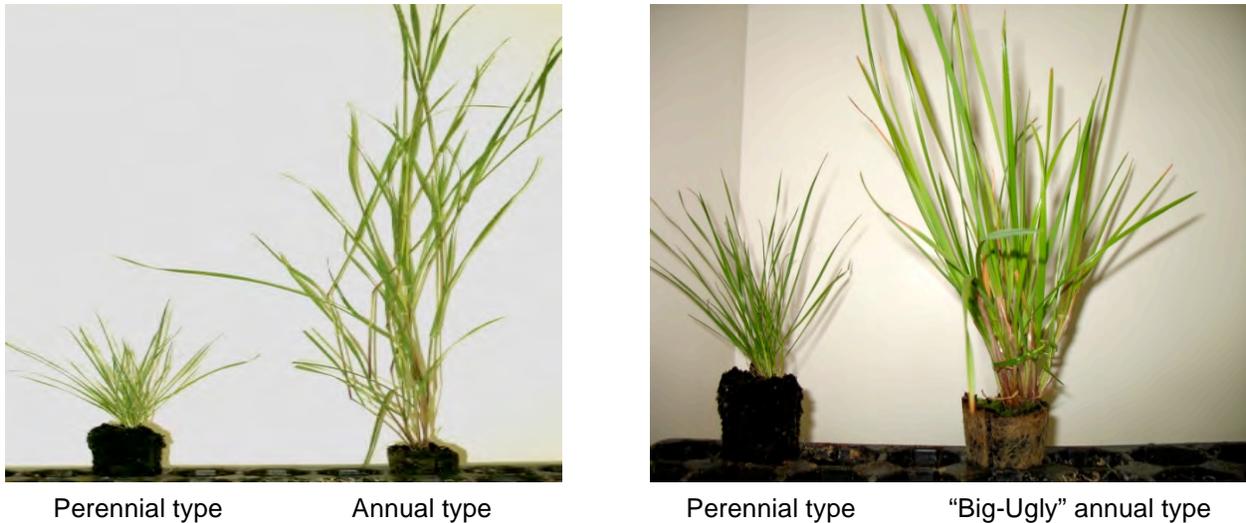


Figure 6. Grow-out test distinguishes between annual, perennial, and “Big & Ugly” plants.

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