FEASIBILITY OF REDUCING THE DURATION OF GERMINATION AND FLUORESCENCE TESTS FOR PERENNIAL RYEGRASS (*LOLIUM PERENNE* L.)

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Introduction

Germination and fluorescence tests are required for labeling certified seed lots of perennial ryegrass. The standard germination test indicates the percentage of viable seeds in a sample, and the fluorescence test quantifies the amount of annual ryegrass contamination in perennial samples which is used to calculate purity test results. The roots of annual type fluoresce upon exposure to UV light, while the roots of perennial type do not fluoresce.

Perennial ryegrass (PRG) is harvested in August and must be shipped to worldwide markets by the end of September in order to arrive on time for fall planting. After seed harvest is completed, the time frame for cleaning, sampling, testing, tagging and shipping is very short. Any delays during this process can lead to missed opportunities for PRG sales.

Germination and fluorescence testing conducted on freshly harvested PRG seeds under the current Association of Official Seed Analysts (AOSA) Rule for Testing Seeds requires pre-chilling treatment at 5-10ºC followed by 14 days of warm germination (15-25ºC). However, previous observations have indicated that the full 14 days of warm germination might not be necessary to achieve maximum germination in all samples and leads to unnecessary delay in delivering final test results. It is true that the chilling period to break dormancy cannot be avoided, however the warm germination period may be shortened. Observations in many labs have indicated that many freshly harvested PRG seeds, when properly chilled, show complete germination in the first count after seven days, and that the resulting seedlings have well-developed roots for full expression of fluorescence (Fig. 1).

In 2011, the AOSA agreed that official evaluation of annual ryegrass (ARG) germination and fluorescent test results could be completed and reported before the 14th day, provided the seed analyst is positive that the sample has reached maximum germination capacity. This change was accepted based on studies, which demonstrated that the majority (over 80%) of ARG samples reach maximum germination and fluorescence at 7 days (first count) on chilled samples (unpublished data by the authors, 2010). This change has allowed seed labs to successfully deliver timely and accurate test results to the seed industry over the last two years, thus, increasing the efficiency of shipping certified ARG seed to global markets. As a result, many seed industry members have asked the Oregon State University Seed Laboratory (OSUSL) to conduct similar studies to determine whether or not germination and fluorescence tests for PRG can be ended at 7-day count rather than enduring the entire 14-day testing period. The potential benefit to PRG would be similar to the improved process utilized for ARG testing and subsequent shipment. Therefore, the objective of this study was to compare the percentage of germination and fluorescence of chilled PRG samples after 7 and 14 days to assess the feasibility of reducing the duration of germination and fluorescence tests for perennial ryegrass.

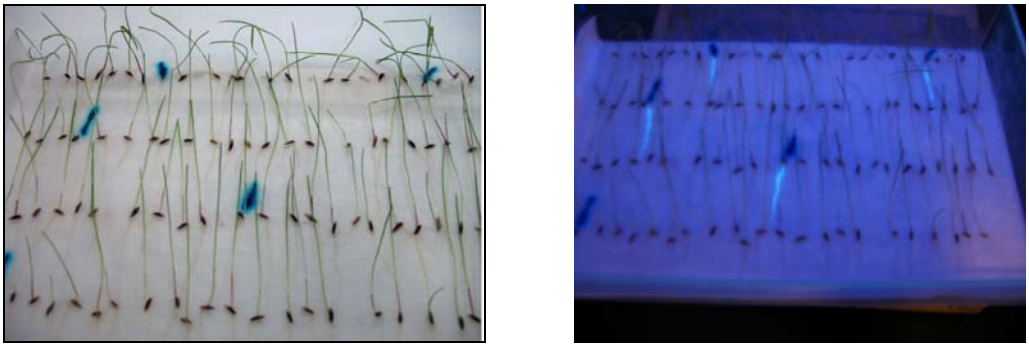

Figure 1. A perennial ryegrass sample showing well developed seedlings and maximum germination capacity and fluorescence expression at the first count (7-day pre-chill + 7-day germination).

Methods

Data from germination and fluorescence test results were collected from the Oregon State University Seed Services database on 2242 PRG samples, representing 203 varieties that were produced and tested in 2011. All samples received pre-chilling treatments before germination.

Samples were germinated according to the AOSA Rules for Testing Seeds, vol. 1. Samples were pre-chilled at 10ºC for 7 days and then transferred to growth chambers for germination at 15-25ºC. Four replications of 100 seeds each were planted for each test. The first germination count was conducted at 7 days and the final count at 14 days. Fluorescence was evaluated at 7 and 14 days as well. Means and standard deviations were calculated to make comparisons between 7 and 14-day test results.

Results and Discussion

Germination Test Results: 7-day versus 14-day

The average germination of the 2242 samples tested in first count (7 days) was 93%, and was 94% at the final count after 14 days (Fig. 2). For practical purpose, these results are comparable and statistically similar. The vast majority of samples had already reached over 90% germination by the first count at 7-days (Fig. 2). This means that the germination level is already above the standard required by certification and meets the needs of most industry customers.

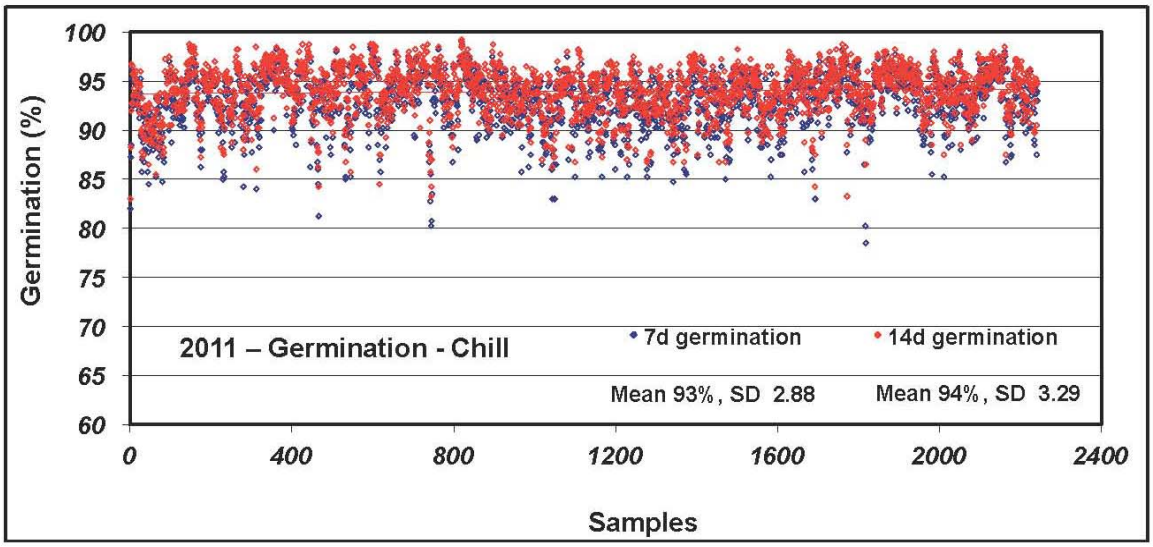

Figure 2. Comparison between 7-day and 14-day germination test results of 2242 perennial ryegrass samples tested with pre-chilling treatments in 2011.

Figure 3 presents the magnitude of change in germination between the first count at day 7 and the final count at day 14. In 35% of the samples, the germination percentage did not increase from the first to the final count. Additionally, 61% of the samples increased only by 1-2%, which is less than the variance that is expected due to random sampling variation. Such variation is negligible for most customers when the germination of the sample is above 90%. These results indicate that 96% of the 2242 samples reached maximum germination first count (7-days) and it was unnecessary to continue testing for an additional 7 days as the current AOSA Rules require.

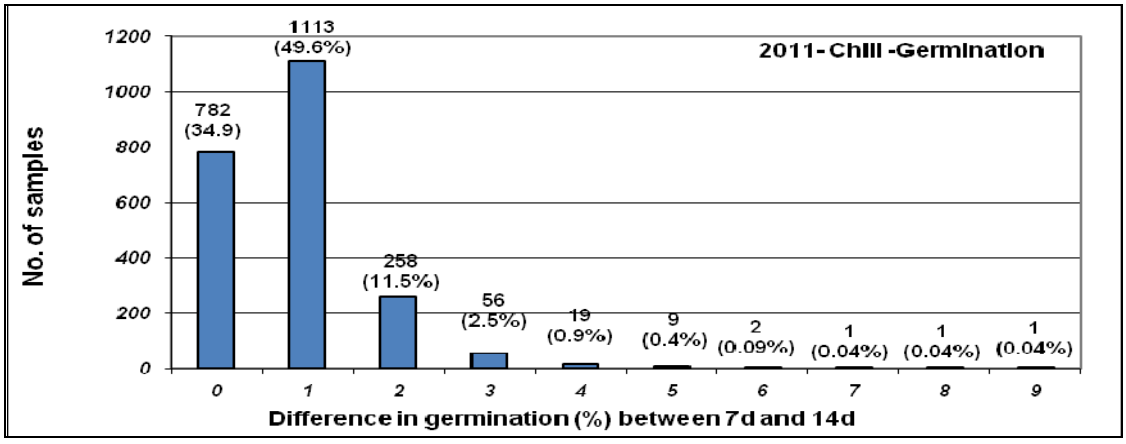

Figure 3. Change in germination test results between the first count at 7 days and the final count at 14 days for 2242 perennial ryegrass samples tested in 2011.

Conversely, in 4% of the 2242 samples the germination results increased by more than 2% between the first and the final counts. Such samples do require the additional 7 days (for a total of 14 day germination) for reach maximum germination. The magnitude of variation between first and final counts depends on varietal differences, environmental influence under which the crop developed and matured, the physiological quality of each seed lot, the age of seeds, and whether seeds were subjected to pre-chilling treatment before the germination test.

Fluorescence Test Results: 7-day versus 14-day

The average difference between the first and the final counts for the fluorescence test results of the 2242 samples did not exceed 0.27% (Fig. 4). This variation in fluorescence is smaller than a typical variation of two subsamples drawn from the same seed lot. It is also worthy to note that the 3% tolerance value for fluorescence in the Oregon Seed Certification Program is greater than this 0.27% difference between the first and final count results detected in this study.

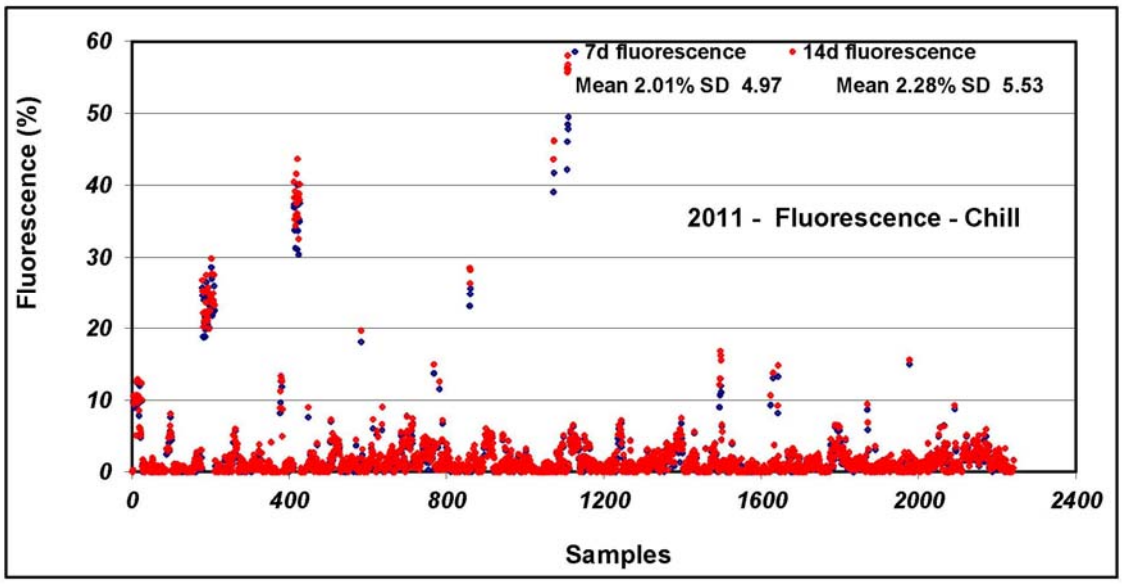

Figure 4. Comparison of fluorescence test results of 2242 perennial ryegrass samples germinated for 7 days and 14 days, with pre-chilling treatment in 2011.

For 54% of the samples, the fluorescence level did not increase from the first to the final count. Additionally, the fluorescence increased only by 1% from the first to the final count in approximately 40% of PRG samples. Overall, the increase in fluorescence level from the first count to the final count did not exceed 1% for a total of 93.5% of all PRG samples tested in 2011 (Fig. 5). Furthermore, for 99% of the total samples tested, the increase in fluorescence level from the first count to the final count did not exceed the 3% tolerance level set by the Oregon certification program (Fig. 5).

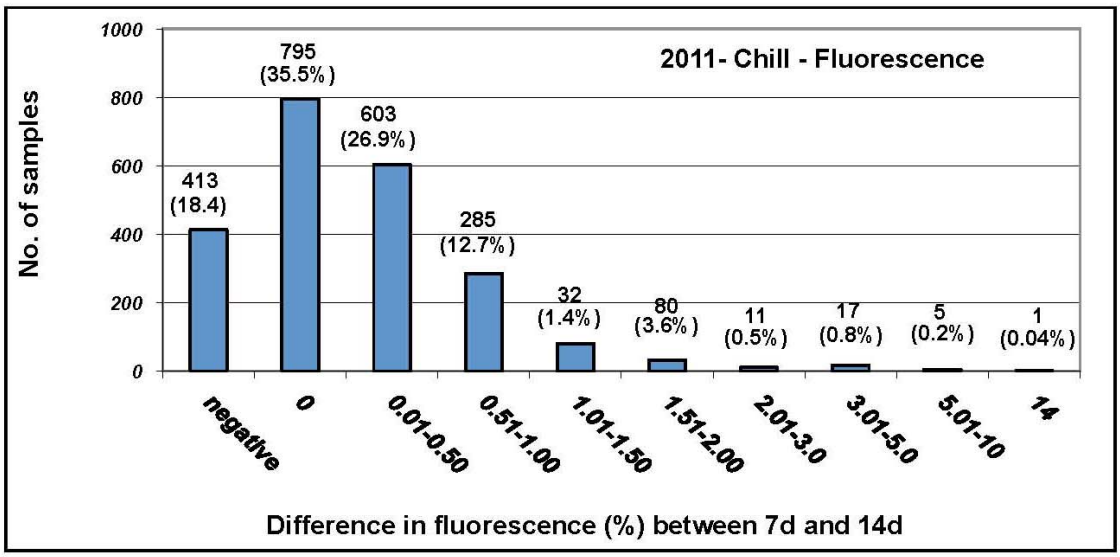

Figure 5. Change in fluorescence test results for 2242 perennial ryegrass samples from the first count at 7 days to the final count at 14 days in 2011.

These results indicate that the expression of fluorescence reached its maximum level at the first count in the vast majority of samples. Generally, if seedlings of annual ryegrass (ARG) developed normal root system after 7 days, they fluoresce upon exposure to UV light and no extra germination time is needed. The roots of ARG possess naturally occurring chemicals called annuoline $[C_{17}H_{10}ON(OCH_3)_3]^2$, a fluorescent pigment that fluoresce when exposed to ultraviolet light. Practical observations also indicate that the change in fluorescence level due to random sampling variation among subsamples from the same seed lot can be more than the changes detected between the first and the final counts in this study. In addition, these changes are smaller than the 3% tolerance allowed by the Oregon Seed Certification Program. The high expression of fluorescence found in this study by the first count can be explained by the high germination that was achieved by first count.

Conclusions

- The majority of freshly harvested PRG samples that have been chilled reached maximum germination and fluorescence by the first count (7-day).
- Sample results that have reached maximum germination by the first count (7-day) can be reported without affecting the final results.
- Samples that did not reach maximum germination in the first count (7-day) should be germinated for full 14 days before ending the test.
- Based on the results, it is appropriate to clarify in the AOSA Cultivar Purity Testing Handbook that ending the germination and fluorescence tests of PRG before 14 days should be allowed, provided the analyst is positive that the sample has reached maximum germination potential.

References

AOSA. 2012. Rules for Testing Seeds. Vol. 1. Assoc. Off. Seed Anal. Ithaca. NY.

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