EXPRESSION OF GERMINATION AND FLUORESCENCE IN ANNUAL RYEGRASS SAMPLES AFTER SEVEN AND FOURTEEN DAY GERMINATION PERIODS

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Introduction

Samples from seed lots of recently harvested annual ryegrass (*Lolium multiflorum* L.) (ARG) possess varying levels of dormancy, depending on the variety and the growing conditions. The Rules for Testing Seeds of the Association of Official Seed Analysts (AOSA) indicate that dormant seeds have to receive pre-chilling treatment to overcome dormancy and allow the seeds to germinate (AOSA, 2012). Samples from seed lots harvested in Oregon in early summer (i.e., early July) are typically chilled through the end of the summer (i.e., September). Starting in early fall, ARG samples are no longer chilled as seed dormancy gradually disappears over time. This occurs through a process known as 'after-ripening' a series of physiological changes that allow dormant seeds to germinate (Copeland and McDonald, 2001).

The AOSA Rules for Testing Seeds indicate that germination tests may end when a sample has reached its maximum germination potential (AOSA, 2012, vol. 1. sec. 6.9d.3). However, in case of ryegrasses, the AOSA Cultivar Purity Testing Handbook (AOSA, 2008) indicates that fluorescence test may not end before the 14-day prescribed germination period regardless of whether or not a sample has reached its maximum germination. It has been observed over years that many ARG samples reach maximum germination after one week of pre-chilling treatment and one week of germination testing at 15-25°C (data not shown). To date, research has not been conducted to systematically evaluate germination and fluorescence patterns exhibited by ARG and determine the feasibility of reducing the germination test period from 14 to 7 days.

The ryegrass seed industry is genuinely concerned about the justification for waiting an additional seven days even if a sample reached maximum germination at the first count (7-days). ARG is usually harvested in July and seeds have to be cleaned, tested, labeled and shipped by the end of August in most cases. This short window of time requires that every activity from cleaning seeds to testing and tagging has to be performed efficiently and effectively, including germination and fluorescence testing. Any delays during this process create missed opportunities for ARG sales. Many members in the seed industry consider the 21-day period currently needed to complete the germination-fluorescence test excessive. It is worthy to note that in 2011 the AOSA agreed that evaluation of ARG germination and fluorescence tests could be completed and reported before the 14th day, as long as the seed analyst is positive that the sample had reached maximum germination. The hypothesis of the study is that if an ARG samples reached maximum germination, it also expresses maximum fluorescence, and waiting additional time will not affect the final results of neither germination nor fluorescence. Therefore, the objectives of this study were to: 1) determine the germination and fluorescence test results of 112 freshly harvested (dormant) ARG samples at 7 and 14 days; and 2) determine the frequency of samples that reach maximum potential germination and fluorescence before 14 days.

Materials and Methods

Data were collected in the summer of 2010 to determine the germination and fluorescence results of 112 freshly harvested seeds of certified seed samples representing 23 ARG varieties. The germination and fluorescence results were collected systematically at 7-day count (first count) and 14-day count (final count) at the Oregon State University Seed Laboratory. Because these samples had been freshly harvested in early summer, they were chilled at 10°C for seven days prior to the warm germination at 15-25°C for 7 and 14 days. All germination and fluorescence tests were conducted according to the AOSA Rules for Testing Seeds (AOSA). For simplicity in representing the research

findings, the fluorescence test results were rounded to whole numbers.

Results and Discussion

Germination Test Results: 7-day versus 14-day

The results of 7 and 14-day germination testing of 112 ARG samples are presented in Figure 1. The mean test results of the 112 samples was 96.16% at the first count after 7 days and was 96.52% at the final count after 14 days. The standard deviation of the 112 samples from their means in the 7-day count was similar at 2.30 in the first count and 2.13 in the final count. The germination data in Figure 1 has been ranked from the highest to the lowest value, based on 7-day count. The results showed that a vast majority of samples expressed high germination, 90% or higher, in the first count, which was similar to that of the final count. Those samples that had achieved high germination in the first count did not change, or had a slight increase between 7 and 14 days counts. This indicates that the majority of samples reached maximum potential germination in the first count and that the germination tests could therefore be ended after 7 days. Leaving the sample in the germinator for extra 7 days did not affect the final results, and may cause an unnecessary delay in test results.

At the lower end of the germination in Figure 1, a few samples showed lower germination results in first count compared to the final count indicating these samples do need to germinate for the full 14-day germination test period to achieve maximum potential 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. It is reported that pre-chilling treatments break dormancy and speed up germination for ryegrass (Elias and Garay, 2008).

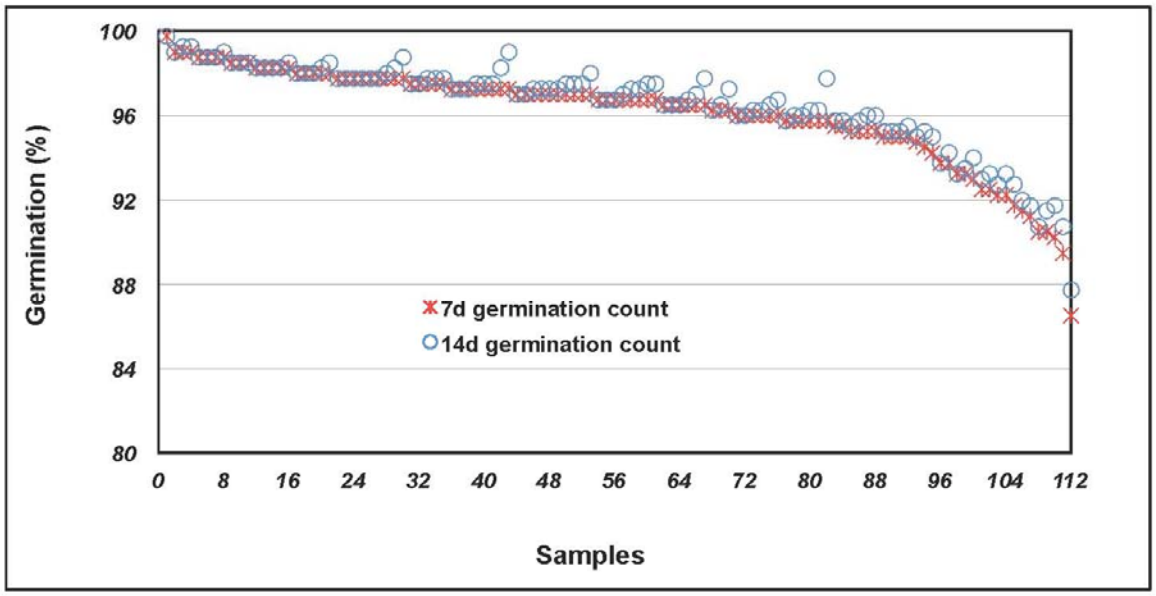

Figure 1. Germination results of 7-day and 14-day counts of 112 ARG samples tested at the OSU Seed Laboratory.

Maximum Germination Frequency: 7-day versus 14-day

The frequency of samples that reached maximum germination in 7 days compared to 14 days is presented in Figure 2. Results showed that a large number of samples had the same germination level in the first and the final counts. In 72 of the 112 samples tested (64%) there was no difference in germination percentage between the first and the final counts (Fig. 2). Those samples had already expressed their maximum germination in the first count. In this case, waiting an extra 7 days did not

improve the final results, but caused unnecessary delay in delivering the results in a timely manner. Likewise, the germination percentage of approximately one-third of the samples increased in the final count (14 days) only by 1% compared to the first count after 7 days (Fig. 2). This increase is minimal and is smaller than the typical random sampling variation of two subsamples drawn from the same seed lot. The germination of a small number of samples (3%) increased in germination in the final count by 2%. This increase is still small, which confirms that most samples achieve maximum germination potential in the first count, and thus the germination tests of those samples could be ended in 7 days rather than 14 days without affecting the accuracy of the results. It is not unusual that 36% of ARG samples did not achieve maximum germination at 7-days. Among the reasons that affect the speed of germination are differences among varieties, growing conditions, dormancy levels, seed vigor, as well as random sampling variation.

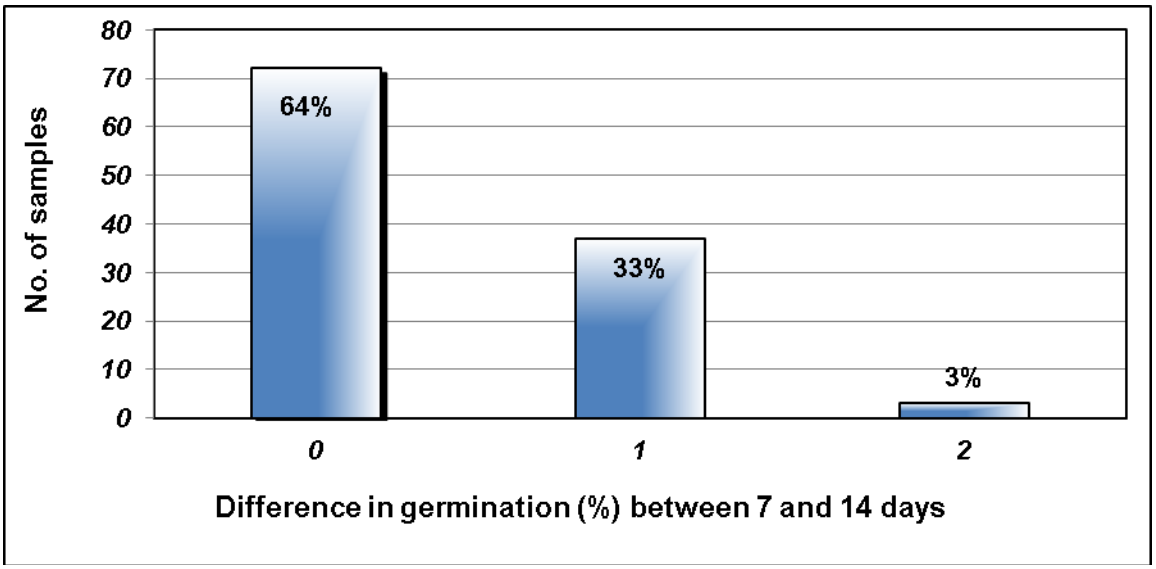

Figure 2. Frequency of samples that reach maximum germination in 7 days compared to 14 days of 112 ARG samples tested at the OSU Seed Laboratory.

Fluorescence Test Results: 7-day versus 14-day.
The fluorescence test results in the first count at day 7 and in the final count at day 14 of the 112 samples are presented in Figure 3. The mean of the 112 samples was 99.31% at the first count after 7 days and was 99.78% at the final count after 14 days. The standard deviations of the 112 samples from their means in both the first and the final counts were small at 1.16 and 0.48, respectively. The fluorescence data has been ranked from the highest to the lowest based on 7-day count (Fig. 3). The results indicated that the vast majority of samples (over 87%) have already expressed the typical high fluorescence of annual ryegrass (i.e., 99-100%) in the first count. Samples that had achieved high fluorescence in the first count either did not increase or slightly increased compared to the final count after14 days. These results indicated that the fluorescence evaluation in such samples could be ended in the first count and that waiting extra seven days would cause unnecessary delay in delivering the results. These results are not surprising because as long as the samples reach maximum germination and developed normal root system, the fluorescence expression is expected to reach full potential. At the lower end of the curve in Figure 3, some samples showed relatively lower fluorescence in the first count compared to the final count, and would therefore need the full 14-day test period before the test is ended.

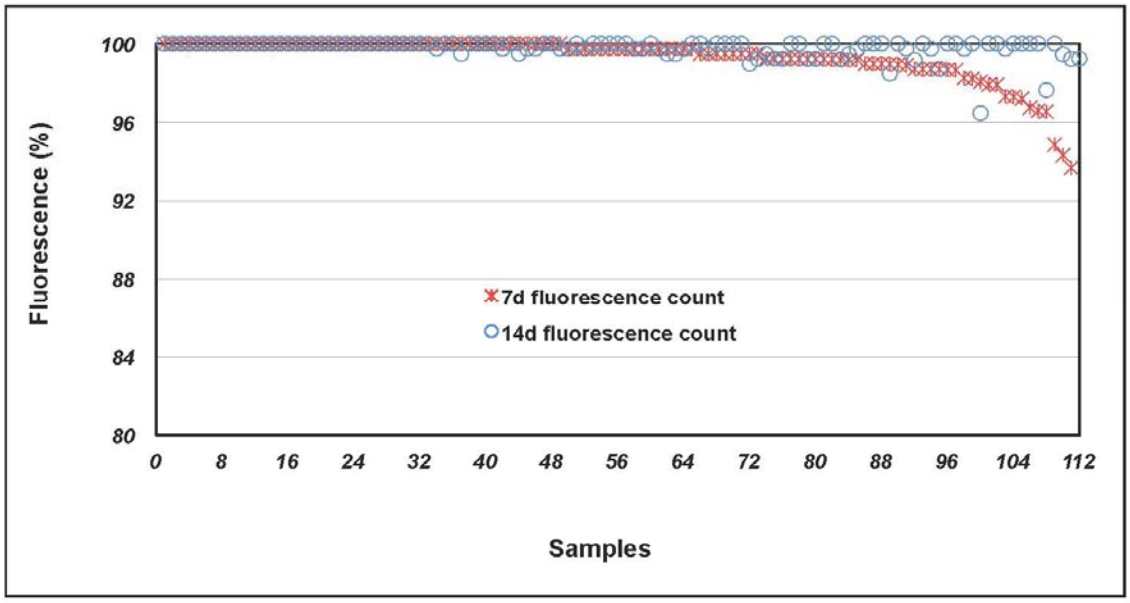

Figure 3. Fluorescence results of 7-day and 14-day counts of 112 ARG samples tested at the OSU Seed Laboratory.

Maximum Fluorescence Frequency: 7-day versus 14-day

The frequency of samples that reached maximum fluorescence in 7 days compared to 14 days is presented in Figure 4. The results indicated that the fluorescence test results of 70% of the 112 samples did not change from the first to the final count, and 19% of the samples changed by only 1% (Fig. 4). The majority of samples (89%) had expressed maximum fluorescence in the first count or had a slight change from the first to the final count. These results indicate that a great number of samples had expressed maximum fluorescence at the first count without the need to extend the test for an additional 7 days.

Feasibility of 7-day Germination and Fluorescence Testing Period.

The results presented in this study confirm previous observations made by germination analysts in many laboratories for several years. These results indicated that over 80% of ARG samples did reach both maximum germination and fluorescence expression in the first count in 7 days and the additional week of test period required by the AOSA rules appears unnecessary. The study also determined that a small percentage of samples increased in germination and fluorescence only by 1% which does not justify waiting an additional 7 days, Such a small increase in germination and fluorescence is insignificant and could be attributed to random sampling variation if the test is to be repeated. These findings indicate the potential for seed analysts to end ARG germination and fluorescence tests when maximum germination is attained at 7-days without compromising accuracy of results. In addition, the results from this study would support a proposed change in AOSA testing protocol, thus, increasing ARG seed testing efficiency by releasing results in a more timely fashion. The small percentage of ARG samples that do not express maximum germination and/or fluorescence in the first count because of dormancy or any other reason do need to germinate for the full 14-day period to make the final evaluation. The seed analyst has the discretion to determine whether a sample reached maximum germination potential on a case-by-case basis.

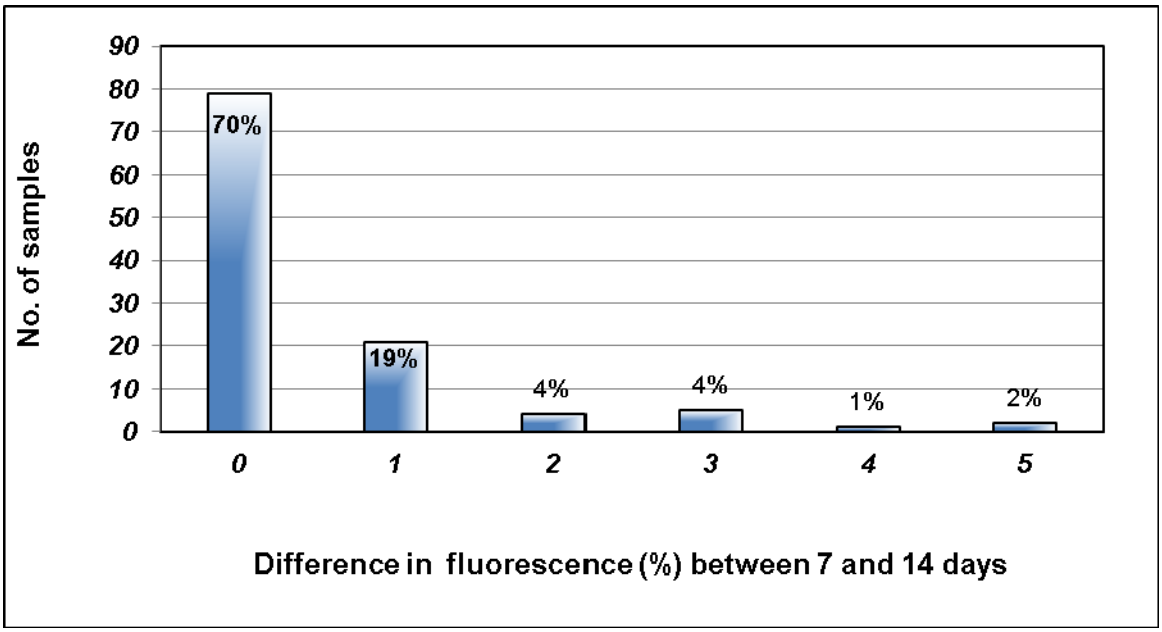

Figure 4. Frequency of samples that reach maximum fluorescence in 7 days compared to 14 days of 112 ARG samples tested at the OSU Seed Laboratory.

Conclusions

- With pre-chilling treatment (7 days at 10°C), the majority of ARG samples reached maximum germination and fluorescence in the first count after 7 days, or changed only by a small magnitude (i.e., 1-2%) after 14 days. The germination and fluorescence tests in such samples could be ended at the first count, thus increasing the timeliness of reporting test results to the industry, without sacrificing accuracy.

- Samples that do not reach maximum germination and/or fluorescence in the first count need to be germinated for the full 14 days before ending the test.

References

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

AOSA. 2008. Cultivar Purity Testing Handbook. Assoc. Off. Seed Anal. Ithaca. NY.

Copeland, L.O. and B.M. McDonald. 2001. Principles of Seed Science and Technology. Springer, NY.

Elias, S.G., and A.E. Garay. 2008. Effect of pre-chilling treatment on the germination and fluorescence of perennial ryegrass. *In* W.C. Young III (ed.), Seed Production Research at Oregon State University, Department of Crop and Soil Science. Ext/Crs 127, 3/08.