Oregon State University Seed Laboratory

2016 Proposal 1 Supporting Evidence

Appendix 1: Discontinue the Factoring Procedure in Fine Fescue Species

Rationale

Factoring procedure is used to estimate the percentage of pure seed and inert matter present in multiple seed units (MSU). Table 3.1 of the AOSA Rules, Vol. 1, has factors for the following fine fescues: creeping red fescue, chewing fescue, hard fescue, and sheep fescue. Each species has a factoring value, and in the case of creeping red fescue, the factor values change depending on the purity level of the sample. The MSU procedure was developed as a means to speed up testing of fine fescues from the days in which all the attached florets had to be physically separated (i.e., broken away) from the single fertile floret. This was a very time consuming testing method that was replaced by the MSU procedure (Meyer, 2001). The proposed method would further simplify and speed up the test procedure.

Factoring is a time consuming procedure because the MSU's have to be separated from the sample (Fig. 1) based on the morphological structure of the florets, which introduces human subjectivity. Afterwards, MSU's have to be weighted separately, and then the percentage of single seed units (SSU) in the sample has to be calculated to determine the proper factor from Table 3.1. Each fine fescue subspecies has different factor value, which has to be applied to the weight of multiples in each sample. The estimated pure seed from MSU's has to be added to the pure seed from SSU portion, and the estimated inert matter from MSU's has to be added to the corresponding inert matter portion in that sample. After all these processes, MSU's are placed back with the pure seed portion for testing the germination of the sample. This whole procedure is cumbersome, slows down purity testing, and needs to be simplified.

Figure 1. Example of separation of a fine fescue sample into pure seed units, inert matter, crop and weed seeds and multiple seed units.

A simpler and more streamlined method would be: "consider all MSU's that contain at least one fertile floret as pure seeds". In this new approach, all SSU's and MSU's that contain a fertile floret(s) would constitute the pure seed portion. This portion would be valid as pure seed and would be ready for germination testing. In addition to simplifying the testing process, this new method would simplify the training and learning process, especially for new analysts and would reduce potential human error in separating, weighing, calculating the factors, and adding to the correct portions.

Another reason to consider discontinuing the factoring procedure is that testing rules followed by International Seed Testing Association (ISTA), which is used in most international markets, does not apply the factoring procedure. Rather, they consider multiple seed units as pure seed units. If the AOSA rules treat MSU's of fine fescue in the same manner as ISTA rules do, more uniformity in test results between the two organizations would be achieved. This would have positive impact on seed lots that are marketed internationally.

It is worthy to note that in 2013, the AOSA accepted a rule proposal to eliminate the factoring procedure in orchardgrass. This change contributed to simplifying the purity testing procedure and reduced subjectivity. In addition, it harmonized AOSA and ISTA rules in regard to multiples in orchardgrass.

Due to the above reasons, several research studies were conducted to explore the possibility of discontinuing the factoring procedure in fine fescues. The first study was conducted at OSU, the second study was conducted among the Northwest seed laboratories with greater number of samples, and the third study was the national referee study with all interested AOSA and SCST Labs in the USA and Canada.

Following are the highlights of these studies.

1. The first study: OSU exploratory study

1.1. Pure seed percentage comparison of fine fescues tested with and without applying the factoring procedure

Two samples of each of creeping red fescue, chewing fescue, hard fescue, and sheep fescue representing different varieties and production years were used in the study. The number of MSU's in samples ranged from 50 to over 300.

The results of the study presented in Figure 2 indicated that the pure seed percentage results without factoring was slightly higher by an average of 0.49% compared to the factoring procedure. For practical purposes, the pure seed percentage results with and without factoring were comparable. Based on these results, it was possible to foresee that, the no-factoring method would be simpler, speed the purity testing process and would produce an appropriate description of the purity of the sample. Additionally, the new method, would describe the purity of the seeds as it is in the bag and the seed lot without creating any distorted perceptions due to mathematical calculations.

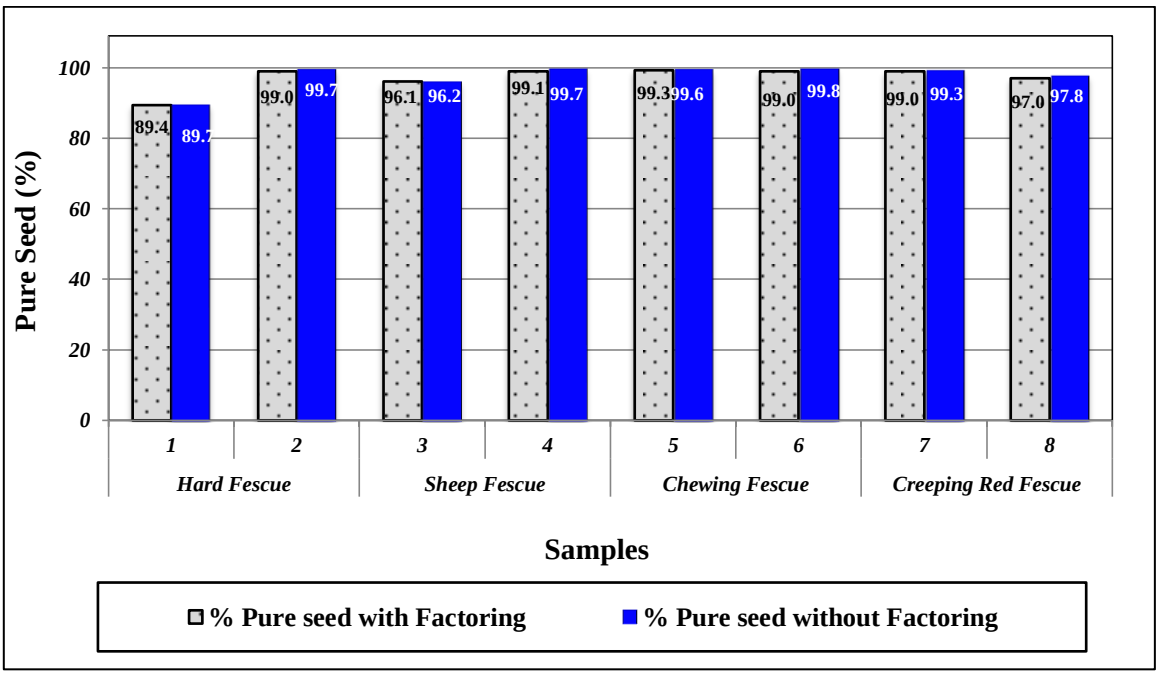

Figure 2. Comparison of pure seed percentage of eight fine fescue samples tested with and without applying the factoring procedure.

1.2. Germination comparison of single seed units and multiple seed units in fine fescues samples

Currently, AOSA rules already includes MSU's as pure seeds for the purpose of germination testing thus the new method would be the same. But, the impact of MSU's on the overall germination of the sample is unknown. Therefore, this study measured the germination of SSU's and MSU's separetly to understand the contribution of each category to the overall germination percentage of a sample.

The same eight fine fescue samples were used in this study. In each sample, 400 seeds were planted from the SSU's, and all MSU's found in each sample, which ranged between 50 to over 300. All samples were chilled at 10°C for 7 days and the germination was conducted at 15-25°C for 14 days.

The germination test results presented in Figure 3 indicated that MSU's have similar germination capacity as SSU's, regardless of the germination level of the sample. Therefore, it is clear that including the MSU's in the pure seed portion, as it is currently done in germination tests, does not have a negative effect on the germination test result of the sample. In addition, the SSU's are the predominant form in any sample (~2300-2400 units) compared to MSU's (100-200 units). Thus, the SSU's determine the germination percentage in any sample. An example of the seedlings produced by SSU's and MSU's is presented in Figure 4.

The purity and the germination results in this study suggested that including MSU's as pure seed units will not have a negative impact either in the purity or the germination test results. Thus, it seems feasible to include the MSU's as pure seed units without the factoring procedure.

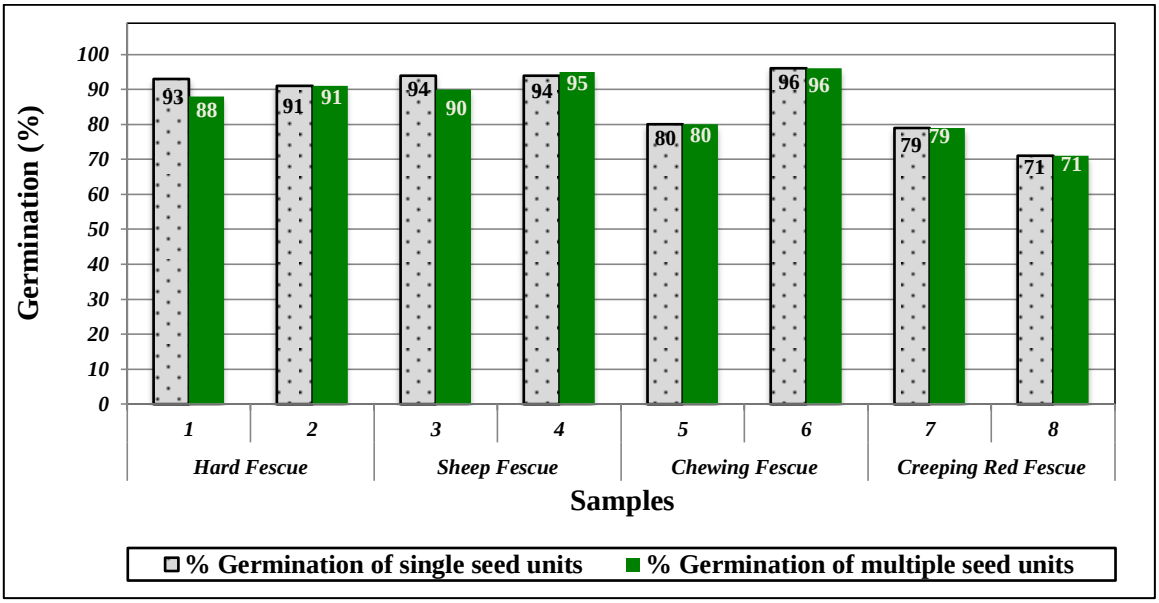

Figure 3. Comparison of single and multiple seed units germination of eight fine fescue samples.

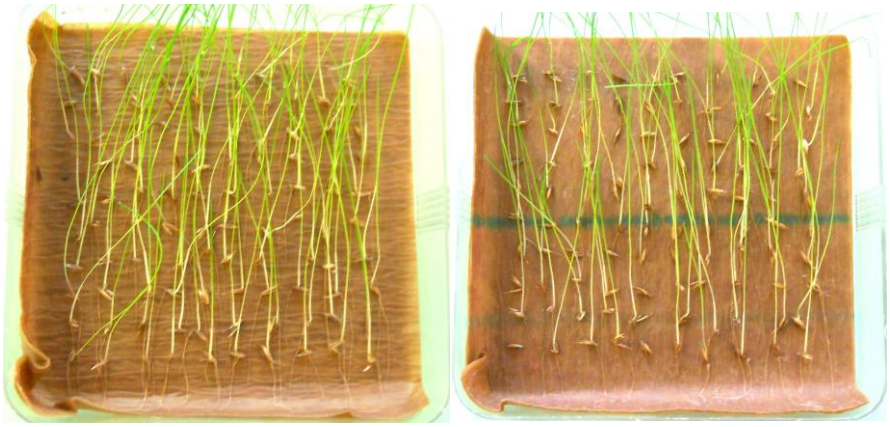

Figure 4. Example of seedlings germination of single seed units (left) and multiple seed units (right) in a fine fescue sample.

2. The second study: Northwest laboratory study

2.1. Pure seed percentage of fine fescues tested with and without applying the factoring procedure

Washington State Department Seed Lab, AgriSeed Testing and Oregon State University Seed Laboratory, labs that test large numbers of fine fescues, participated in this study . The study included a total of 659 samples: 145 hard fescue, 88 sheep fescue, 159 chewings fescue, and 267 creeping red fescue, tested with and without applying the factor procedure. Data that had been collected at these labs for 2-3 previous years were made available for this study.

The average increase in pure seed percentage of the 659 samples was 0.53% when the factoring procedure was not applied. The average increase within each species was: 0.38% for hard fescue;

0.45% for sheep fescue; 0.63% for chewing fescue and 0.67% for creeping red fescue. Similar trend was observed for all fine fescue subspecies; hence only one example is presented in Figure 5.

These results confirmed the previous results obtained by the exploratory research at OSU (Study 1), and provided a higher level of confidence because of the large number of samples used in the study.

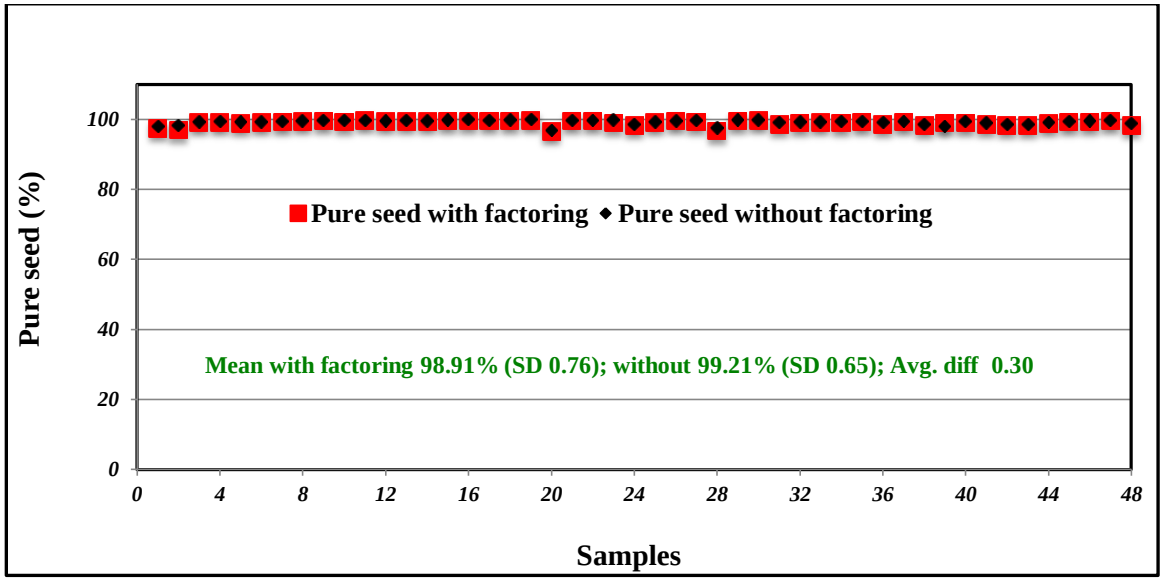

Figure 5. Pure seed percentage of 48 creeping red fescue samples tested in 2010 with and without factoring procedure at AgriSeed Testing.

3. The third study: Natinal Referee

3.1. Pure seed percentage comparisons of fine fescues tested with and without applying the factoring procedure

Nine laboratories participated in the national referee. Some laboratories tested two sets of samples, thus a total of 11 sets of samples were tested. The laboratories received blind samples and were asked to test for purity with and without applying the factoring procedure. A total of 8 samples: two each of red creeping, chewing, hard and sheep fescues were provided to each laboratory. The pure seed percentages with and without factoring of each sample were compared across laboratories on a sample by sample basis.

Since similar results were obtained for all samples, only the results of the first sample set (out of the 8 samples tested in each lab) is presented (Fig.6). The results showed that pure seed percentages without applying the factoring procedure were slightly higher compared to applying the factoring in all labortories (Fig. 6). The average increase in pure seed percentage across samples and labs was 0.58%. This is consistent with the results of the exploratory research (0.49% increase) and the northwest studies (0.53% increase). Based on these three studies, it is possible to conclude that the new method will increase the pure seed percentage of the sample but the increase will not be significant. For practical purposes, this magnitude of the change in purity is the same or smaller

than the typical variation due to random sampling variation. Furthermore, the consistency of the results across laboratories were similar in both methods.

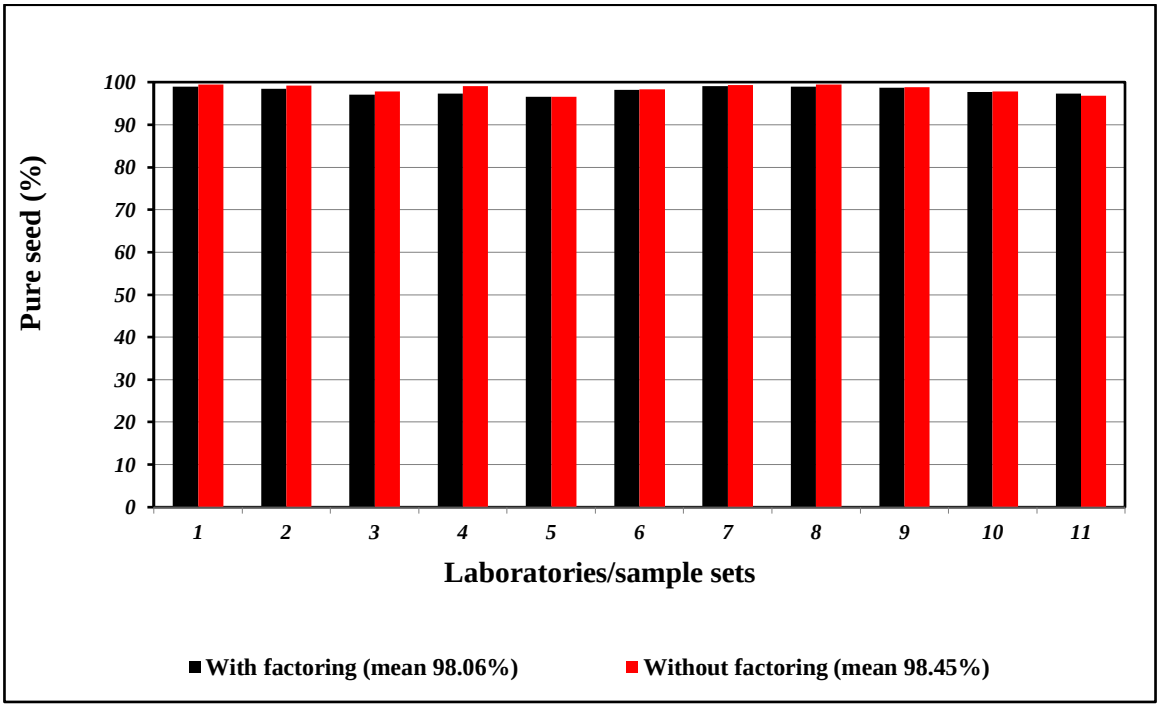

Figure 6. Comparison of pure seed percentage of red creeping fescue sample tested in 11 laboratories with and without applying the factoring procedure.

3.2 Time comparisons

One of the problems with the factoring method is that it takes extensive time. Thus, the labs that participated in the national referee were asked to measure the time taken by each method (with and without factoring) for each sample.

The average time for each laboratory and each testing method across all samples is presented in Table 1.. The results indicate that the overall average time saved with the new method (across samples and laboratories was about 15 minutes or 41% time saving). The time recorded by laboratory number 6 appered to be drastically shorter than all other labs, but it was included in the averages beause their purity results were comparable to the other labs.

The time reduction when the new method was applied is significant for several reasons: It will allow labs to provide more timely results to its customers and it will allow labs to save on cost of labor. These factors are critical for labs that test hundreds of samples of these species each year in producing states.

Table 1. Average time to complete purity test of fine fescue in different labs using factoring and without factoring procedures. Each data point is an average of 8 samples.

Labs	Factoring	
	With	Without
	Time (min)	
1	44.9	24.1
2	50.6	24.8
3	57.5	35.6
4	50.9	21
5	23.4	17.8
6	7.9	4.5
7	32.3	19.9
8	31.5	18.9
9	14.8	9.8
10	48.1	38.3
11	29.5	14.1
Mean	***35.6***	***20.8***

Conclusions

- The new method (non-factoring method) makes an adequate representation of the seeds as they are in the bag and planted. Any planting unit (single or multiple) that contains at least one seed is considered pure seeds for purity testing as well as for germination testing.

- The new method would increase the pure seed percentage. This research showed an average increase of 0.53% but the specific change would depend on the specific sample.

- The MSU's in fine fescues have similar germination capacity as SSU's. Including MSU's in the pure seed portion (as it is already done and will continue to be done) will not have a negative effect on the germination of the sample.

- Conducting purity testing without applying the factoring procedure contributes to time saving, which is important in order to deliver timely results and reduce the cost of processing a sample.

- Eliminating the factoring procedure would harmonize AOSA with ISTA procedures in regard to the MSU's. This would be beneficial for USA seed producers who export seeds.

- The findings of these studies were presented to the Fine Fescue Commission in Oregon (growers), the Oregon Seed Association (trade) and the Oregon Seed Services Advisory

Committee of the Oregon Seed Council (all seed sectors) and received unanimous support.

References

Meyer, D. J. L. 2001. Comparison of four purity testing methods for *Festuca brevipila* R. Tracey and *F. ovina* L. Seed Technology 23(1):35-49.

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