# Rule Change Proposal No. 11

## PURPOSE OF PROPOSAL

Change the pure seed definition number 21 for *Lolium* spp., *Festuca arundinacea*, and *F. pratensis* to achieve two critical and complementary objectives:

1. Eliminate the need to "dismember" the multiple seed unit. Such dismembering artificially creates an inert matter that does not exist in the seed lot. The new pure seed definition will better comply with the AOSA definition of a seed unit as the "structure usually regarded as a seed in planting practices and in commercial channels", hence the Purity report will more accurately reflect the actual purity of the seed lot.

2. Eliminate from the pure seed fraction those seed units with caryopses less than one-third the length of the palea. The current definition "some degree of endosperm development… " is difficult to be standardized. This procedure would be less subjective, more uniform, faster, and would harmonize with the pure seed definition used by ISTA.

Adopting these changes will increase testing objectivity and repeatability, reduce variation between analysts and laboratories, reduce the time required for purity testing of these species, and reduce discrepancies in purity testing results between AOSA and ISTA.

## PRESENT RULE

*PSU Number 21*.

Floret with attached empty floret(s) not extending to the tip of the fertile floret (excluding the awn), or single floret, provided a caryopsis with some degree of endosperm development can be detected (either by slight pressure or by examination over light).
Caryopsis or piece of broken caryopsis larger than one-half of the original size.
Special consideration:

- A fertile floret attached to another fertile floret shall be separated.
- Attached glumes and empty florets extending to or beyond the tip of the fertile floret shall be removed and classified as inert matter.

## PROPOSED RULE

*PSU Number 21*.

- Multiple floret spikelet, multiple floret, or single floret, with or without pedicel, with or without awn, provided there is a caryopsis at least one-third the length of the palea measured from the base of the rachilla.
- Caryopsis or piece of broken caryopsis larger than one-half of the original size.

*Special consideration*: Multiple seed units that contain a fertile floret (with a caryopsis at least one-third the length of the palea) shall be left intact and included in the pure seed fraction.

## HARMONIZATION STATEMENT

This proposal would harmonize the PSU definition for *Lolium* spp., *Festuca arundinacea*, and *F. pratensis*. with ISTA Rules and facilitate the global seed trade of these crops. The proposed pure seed definition of the above species differs from the current pure seed definition in the FSA which is "Caryopses and single florets" [section 201.47a (b)1], "if a caryopsis with some degree of endosperm development can be detected in the units, either by slight pressure or by examination over light" [section 201.48 (g)].

## SUPPORTING EVIDENCE

See attachments, which include the following:

*a) Supporting evidence for including the MSU in the pure seed fraction*:

Elias, S, A. Garay, L. Schweitzer, and S. Hanning. 2002. Evaluation of multiple seed units of perennial ryegrass and tall fescue in purity testing.

Elias, S., S. Davidson, and Meyer, D.. 2002. Purity analysis comparative study of two methods dealing with multiple seed unit in *Lolium* spp. and *Festuca* spp. AOSA Purity Subcommittee.

Meyer, D. L. 2000. Comparison of Two Methods of Purity Testing for Festuca pratensis Hadson and Lolium x hybridium Haussknecht. Seed Technol. 22: 59-63.

b) *Supporting evidence for using seed with caryopsis at least one-third the length of the palea in PSD:*

Elias, S., and A. Garay. 2002. Germination of perennial ryegrass seeds with caryopsis less than one-third the length of the palea.

Elias, S., Hanning, S., and A. Garay. 2002. Effect of single florets with caryopsis less than one-third the length of the palea on pure seed and inert matter components of ryegrass and tall fescue.

Everson, L. E. 1954. The germination of mature and immature seeds of quackgrass (*Agropyron repens*). Proc. Assoc. Off. Seed Anal. 44: 127-128.

Jane Penrose. 1997. Tall fescue germination of caryopsis less than one-third the length of the palea.

## SUBMITTED BY

- Oregon State University Seed Laboratory: Adriel Garay, Sabry Elias, and Sherry Hanning Seedlab@oregonstate.edu Ph (541) 737-4464

- Purity Subcommittee, Deborah Meyer dmeyer@cdfa.ca.gov Ph (916) 262-1137

**DATE SUBMITTED:** October 10, 2002 Revised January 9, 2003

**If proposal 11 is defeated then each item contained within Proposal 11 will be voted on independently as stated in Proposals 11a and 11b.**

# Rule Change Proposal 11a

## PURPOSE OF PROPOSAL

Change the pure seed definition number 21 for *Lolium* spp., *Festuca arundinacea*, and *F. pratensis*. The proposed rule will eliminate the need to "dismember" the multiple seed unit. Such dismembering artificially creates an inert matter that does not exist in the seed lot. The new pure seed definition will better comply with the AOSA definition of a seed unit as the "structure usually regarded as a seed in planting practices and in commercial channels", hence the Purity report will more accurately reflect the actual purity of the seed lot.

Adopting these changes will increase testing objectivity and repeatability, reduce variation between analysts and laboratories, reduce the time required for purity testing of these species, and reduce discrepancies in purity testing results between AOSA and ISTA.

## PRESENT RULE

*PSU Number 21*.

Floret with attached empty floret(s) not extending to the tip of the fertile floret (excluding the awn), or single floret, provided a caryopsis with some degree of endosperm development can be detected (either by slight pressure or by examination over light).
Caryopsis or piece of broken caryopsis larger than one-half of the original size.
Special consideration:

- A fertile floret attached to another fertile floret shall be separated.
- Attached glumes and empty florets extending to or beyond the tip of the fertile floret shall be removed and classified as inert matter.

## PROPOSED RULE

*PSU Number 21*.

Multiple floret spikelet, multiple floret, or single floret, with or without pedicel, with or without awn, provided a caryopsis with some degree of endosperm development can be detected (either by slight pressure or by examination over light).

Caryopsis or piece of broken caryopsis larger than one-half of the original size.

*Special consideration*: Multiple seed units that contain a fertile floret shall be left intact and included in the pure seed fraction.

**SUPPORTING EVIDENCE**

See attachments, which include the following:

Elias, S, A. Garay, L. Schweitzer, and S. Hanning. 2002. Evaluation of multiple seed units of perennial ryegrass and tall fescue in purity testing.

Elias, S., Hanning, S., and A. Garay. 2002. Effect of single florets with caryopsis less than one-third the length of the palea on pure seed and inert matter components of ryegrass and tall fescue.

Elias, S., S. Davidson, and D. Meyer. 2002. Purity analysis comparative study of two methods dealing with multiple seed unit in Lolium spp. and Festuca spp. AOSA Purity Subcommittee.

Meyer, D. L. 2000. Comparison of Two Methods of Purity Testing for Festuca pratensis Hudson and Lolium x hybridium Haussknecht. Seed Technol. 22: 59-63.

**HARMONIZATION STATEMENT**

This proposal would harmonize the PSU definition for *Lolium* spp., *Festuca arundinacea*, and *F. pratensis*. with ISTA Rules and facilitate the global seed trade of these crops. The proposed pure seed definition of the above species differs from the current pure seed definition in the FSA which is "Caryopses and single florets" [section 201.47a (b)1], "if a caryopsis with some degree of endosperm development can be detected in the units, either by slight pressure or by examination over light" [section 201.48 (g)].

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**DATE SUBMITTED:** October 10, 2002 Revised January 9, 2003

## Rule Change Proposal 11b

**PURPOSE OF PROPOSAL**

Change the pure seed definition number 21 for *Lolium* spp., *Festuca arundinacea*, and *F. pratensis*. It will eliminate from the pure seed fraction those seed units with caryopses less than one-third the length of the palea, and avoid the subjective interpretation of "some degree of endosperm development… ", which would result in reducing variation between analysts and laboratories. Adopting this change will reduce the time required for purity testing of these

species. Furthermore, seeds that are less than one-third the length of the palea do not have the capacity to germinate and should be considered inert.

Adopting this change will increase testing objectivity and repeatability, reduce variation in test results, reduce the time required for purity testing of these species, and reduce discrepancies in purity testing results between AOSA and ISTA.

**PRESENT RULE**

*PSU Number 21*.

Floret with attached empty floret(s) not extending to the tip of the fertile floret (excluding the awn), or single floret, provided a caryopsis with some degree of endosperm development can be detected (either by slight pressure or by examination over light).
Caryopsis or piece of broken caryopsis larger than one-half of the original size.
Special consideration:

- A fertile floret attached to another fertile floret shall be separated.
- Attached glumes and empty florets extending to or beyond the tip of the fertile floret shall be removed and classified as inert matter.

**PROPOSED RULE**

*PSU Number 21*.

Floret with attached empty floret(s) not extending to the tip of the fertile floret (excluding the awn), or single floret, provided there is a caryopsis at least one-third the length of the palea measured from the base of the rachilla.
Caryopsis or piece of broken caryopsis larger than one-half of the original size.
Special consideration:

- A fertile floret attached to another fertile floret shall be separated.
- Attached glumes and empty florets extending to or beyond the tip of the fertile floret shall be removed and classified as inert matter.

**HARMONIZATION STATEMENT**
This proposal would harmonize the PSU definition for *Lolium* spp., *Festuca arundinacea*, and *F. pratensis*. with ISTA Rules and facilitate the global seed trade of these crops. The proposed pure seed definition of the above species differs from the current pure seed definition in the FSA which is "Caryopses and single florets" [section 201.47a (b)1], "if a caryopsis with some degree of endosperm development can be detected in the units, either by slight pressure or by examination over light" [section 201.48 (g)].

**SUPPORTING EVIDENCE**

Elias, S., and A. Garay. 2002. Germination of perennial ryegrass seeds with caryopsis less than one-third the length of the palea.

Elias, S., Hanning, S., and A. Garay. 2002. Effect of single florets with caryopsis less than one-third the length of the palea on pure seed and inert matter components of ryegrass and tall fescue.

Everson, L. E. 1954. The germination of mature and immature seeds of quackgrass (*Agropyron repens*). Proc. Assoc. Off. Seed Anal. 44: 127-128.

Jane Penrose. 1997. Tall fescue germination on caryopsis less than one-third the length of the palea.

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**DATE SUBMITTED:** October 10, 2002 Revised January 9, 2003

**OREGON STATE UNIVERSITY SEED LABORATORY**

# Evaluation of Multiple Seed Units
## of Perennial Ryegrass and Tall Fescue in Purity Testing

***Sabry Elias, Adriel Garay, Lee Schweitzer, and Sherry Hanning***

## INTRODUCTION

There are three methods to deal with the multiple seed units 'MSU' (also called multiple florets) in the purity testing of grasses:

1. The current AOSA method which requires pulling off all the multiple florets from each sample, breaking them apart, separating them into pure seed and inert matter, weighing the components, and calculating the purity accordingly. This process is cumbersome and creates problems for both seed testing and grass seed industry. It is not uncommon that testing one sample may take over one hour. If the time spent in pulling off multiples does not contribute to accuracy, it adds unnecessary cost to each sample, takes away time and other resources that can be directed to other crucial purity testing activities, and increases the testing backlog during busy seasons. Perennial ryegrass (PRG), *Lolium perenne*, and tall fescue (TF), *Festuca arundinacea* are examples for grass species that have to follow the AOSA rules in dealing with the MSU.

2. The factor method, which is used by AOSA to mathematically determine the proportion of pure seed and inert material of the MSU in some grass species such as orchardgrass, hard, creeping, chewing, red and sheep fescues, smooth brome and 5 different wheatgrass species (AOSA Rules for Testing Seeds, 2000). This method requires physical separation and weighing of multiples of each sample, then calculating the pure seed and inert according to an established factor. This process takes time and some errors and/or variation may associate with it.

3. Method 3, leaving the MSU intact and include them in the pure seed fraction. This is similar to ISTA method, which considers multiple florets of PRG and TF and all other grass species as standard seed units and no longer requires reporting the percentage of their occurrence

unless the customer requests the information. This method is fast, does not require separation, and reduces variability in purity testing among labs due the MSU.

Considering the current global competitive market of grass seed industry, there is an urgent need for research to reevaluate the current AOSA lengthy method and develop a faster, simple method to deal with the MSU in purity testing of PRG and TF while keeping the accuracy level comparable to the current method.

This research project explored the feasibility of adopting method 3 and evaluated the effect of each of the above methods on the speed and accuracy of purity testing results of PRG and TF.

## OBJECTIVES

1. Determine the magnitude of multiple florets in PRG and TF from randomly selected samples that were submitted to OSU Seed Lab in 2001 season.
2. Develop a factor method for PRG and TF (similar to the current AOSA factors for orchardgrass, hard, sheep, chewing, red and creeping fescues, etc.) to determine the percentage of pure seeds and inert matter in the multiple florets of PRG and TF. This was an initial goal for this project.
3. After preliminary research and discussion with other Oregon seed labs, a more ambitious goal was set to reevaluate the current AOSA method of separating the MSU in PRG and TF and compare it with method 3 (which consider multiple florets as part of pure seed fraction).

## *EXPECTED IMPACT*

Develop a simpler, more efficient, repeatable method of dealing with the multiple florets in PRG and TF that would achieve the following:

1. Speed up purity testing of PRG and TF and provide faster results to the seed industry.
2. Reduce variation among labs and analysts due to differences in skills and techniques of pulling off, breaking and separating MSU from samples in the current AOSA method.
3. Keep the integrity of multiple florets, which is regarded as seed units in planting practices and in commercial channels.

**Two studies have been completed to achieve the above objectives:**

1. ***Oregon State University Seed Laboratory Study:*** to investigate the frequency of MSU occurring in PRG and TF.
2. ***The National Referee Study:*** to measure the proportion of pure seeds and inert mater in the MSU of PRG and TF and develop a factor method for these two species.

***Data from both studies were used to explore the feasibility of adopting method 3 for dealing with the MSU in purity testing of PRG and TF.***

## *SUMMARY OF RESEARCH PROCEDURES*

### *1. Oregon State University Seed Laboratory Study*

The following procedures were conducted to determine the magnitude of MSU in random PRG and TF samples of 2001 season, and to assess potential time saving using method 3 (i.e., include the MSU in the pure seed fraction):

- 100 random samples each of PRG and TF from 2001 crop were used in this study.
- MSU were separated from each purity-working sample (minimum 5 grams) using magnifying lens.
- The total weight of each working sample and the MSU content in grams was recorded and the percentage of the MSU in each sample was calculated.
- Further separation of the MSU in each sample into pure seeds and inert matter (i.e. sterile florets, stems, glumes, etc) was made and the weight in gram of each component was recorded.
- The time in minutes required to make the above separation was recorded for each sample.
- All the data were subjected to appropriate statistical analysis to determine the extent of the MSU in the 100 samples each of PRG and TF.

### *2. The National Referee Study*

A national referee study was coordinated by the Oregon State University Seed Laboratory to measure the pure seed portion in the MSU of various PRG and TF samples representing different cultivars and levels of MSU and develop a factor method for PRG and TF. The following procedures were conducted

- Twenty randomly selected seed samples from 2000 season of each of PRG and TF representing 18 cultivars of PRG and 17 of TF were used in the study. MSU in these samples ranged from1.18 to 9.64% in PRG and from 0.11 to 8.57% in TF.
- Five-gram working samples from each lot were prepared according to the AOSA procedure and randomly sent to participating labs with experience in testing PRG and TF.
- Official and private seed laboratories from OR, CA, WA, MN, VA, SD, TX, GA, MO, MI, NY, and CO participated in this study.
- All labs were asked to:

  a) Separate each working sample into single seed units (SSU), free inert materials (other than those from MSU), and multiple florets as defined in the 2000 AOSA Rules for Testing Seeds, and record the weight in grams of each component.
  b) Make further separation of the MSU in each sample into pure seed and inert matter and record the weight in grams of each component separately.

- The time in minutes required to make the above separation for each sample was recorded.
- Fourteen labs completed the test results, seven for each of the PRG and TF samples and submitted the data to Oregon State Seed Lab for analysis. Six labs did not submit any results and three submitted data but was not reported according to the standard data sheet of the study, hence could not be used.
- All the data were subjected to appropriate statistical analysis to determine the constant factor that can be used to calculate the percentage by weight of pure seed in multiple florets of PRG and TF.

***To achieve the third objective:*** Data that had been collected from both OSU Seed Laboratory study (with 100 seed lots of each PRG and TF) and from the national referee study (with 20 seed lots of each PRG and TF) were analyzed to compare the percentage of pure seeds in PRG and TF using the AOSA method and method 3 (i.e., similar to ISTA method). The time saving using method 3 was compared to the current AOSA method.

## *RESULTS AND DISCUSSION*

### *A. The magnitude of multiple florets in perennial ryegrass and tall fescue*

The frequency analysis of Oregon State Seed Lab study showed that 62% of the 100 PRG samples had less than 4% MSU, and 83% had less than 6% MSU (Fig. 1). The results also showed that 80% of the 100 TF samples had less than 2% MSU, and 95% of samples had less than 4% (Fig. 2).

Out of the 100 PRG samples tested, only 2 had over 10% MSU (10.57 and 12.59%). The mean MSU of the 100 PRG samples was 4.04% ranging from 0.86 to 12.59%. The results also showed that the magnitude of MSU in the 100 TF samples was much less than in PRG with a mean of 1.54% ranging from 0.11 to 7.16%. Furthermore, only 5 samples out of the 100 TF samples had MSU of 4.0% or above with a maximum of 7.16% (Table 1 and Figures 1 and 2). Similar MSU frequency was observed in the national referee study in both PRG and TF. The mean MSU of the 20 PRG samples was 3.38 (Table 2) ranging from 1.18 to 9.64%, whereas the mean MSU of the 20 TF samples was 1.87(Table 3) ranging from 0.11 to 8.57%. This low MSU content in PRG and TF, in part, can be attributed to the technological advancement in seed cleaning machinery and to the efforts of each grower to provide high quality seeds in today's competitive market. Personal communication with county agents confirmed that MSU content in seed lots does not impose constraints on mechanical planting (i.e., does not block the holes in the drill).

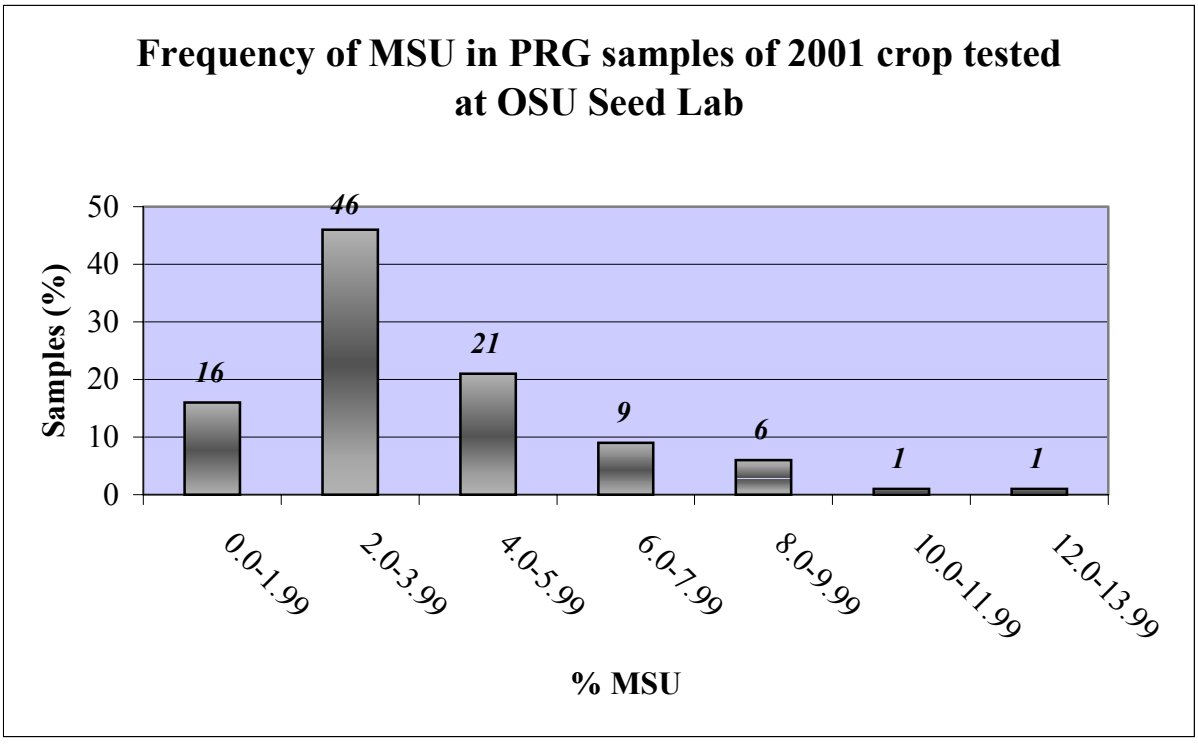


***Fig. 1***

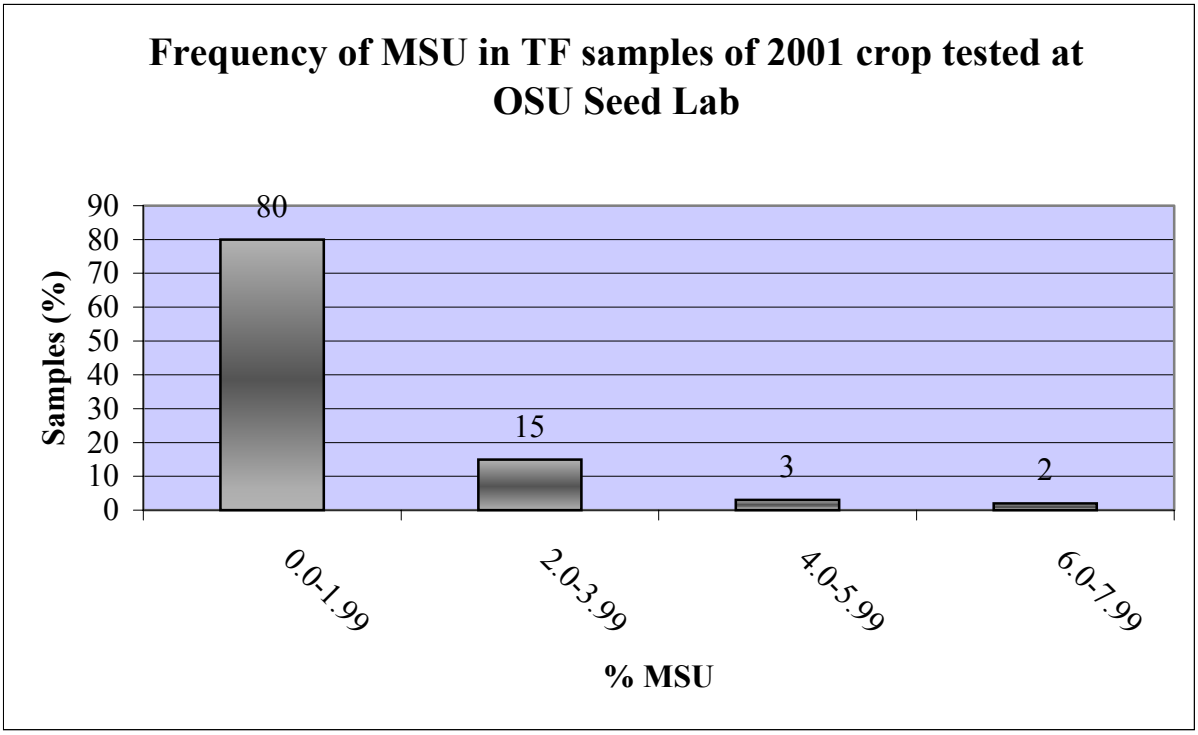


***Fig. 2***

***B. Determine the percentage of pure seed and inert in MSU of PRG and TF***

Fourteen participating labs from the national referee study submitted the complete requested data, seven for each crop. The data included the percentage of MSU, single seed units (SSU), pure seed in MSU, inert in MSU, free inert (not from MSU), the time spent to separate and break down MSU into pure seed and inert in each sample, the total pure seed as measured by the current AOSA method, and by method 3, which is similar to ISTA method in considering the multiple florets as seed units (Tables 2 and 3). The pure seed content in the MSU of PRG and TF was calculated and used as the constant factor for mathematically estimating pure seed in MSU of these crops. This factor was found to be 0.72 in PRG and 0.71 in TF crops (Figs. 3 and 4). For example, if a working purity sample of PRG or TF (5 grams) has 5% MSU weighing 0.300g; only 0.084g in PRG and 0.087g in TF is inert matter and the rest of the 0.300g is pure seed. This represents a small portion of inert materials in the whole 5-gram sample. In general, the total pure seed percentage of the 20 samples across the seven labs was 97.50 for PRG and 97.54 for TF as measured by the AOSA and was 98.45 and 98.08, respectively as measured by method 3 (Table 2 and 3).

The inert from MSU represented small percentage of the total weight in both crops, 0.95 in PRG and 0.54 in TF compared to the free inert (not from the MSU), which was 1.55 in PRG and 1.94 in TF (Figs 3 & 4). If method 3 were applied, the percentage of inert from MSU (0.95 in PRG and 0.54 in TF) would be added to the pure seed fraction of each sample, whereas it would be added to the inert fraction in the current AOSA method.

The results showed that as the MSU level decreased in PRG or TF samples, the difference between method 3 and AOSA method also decreased. For example, in PRG when the MSU level was 1.18%, the difference in the total pure seed percentage using method 3 and AOSA method was only 0.39%. This means if the total pure seed in a sample was found to be 98% using AOSA method, it would be 98.39% if method 3 was applied. However, when the MSU content was 9.64%, the pure seed percentage was 2.79% higher using method 3 than AOSA method. Similar trend was observed in TF (data not shown in tables).

***Table 1. Percentage of multiple seed units and pure seeds as determined by AOSA and Method 3 for 100 PRG and 100 TF samples of 2001 crop tested at OSU Seed Lab.***

| Crop | Samples ***1-100*** | MSU | ***Total Pure Seeds by*** | | Diff. Method3 minus AOSA | Time saved by method 3 |
|---|---|---|---|---|---|---|
| | | | ***AOSA method*** | ***Method 3*** | | |
| | | ***%*** | ***%*** | | ***%*** | ***min*** |
| ***PRG*** | ***Mean*** | ***4.04*** | ***97.67*** | ***98.54*** | ***0.87*** | ***63*** |
| TF | ***Mean*** | ***1.54*** | ***98.49*** | ***98.91*** | ***0.42*** | ***41*** |

**Table 2. Mean Percentage of Sample Components Including Multiple Seed Units (MSU), Pure Seed and Inert Matter in 20 PRG Samples Tested at 7 Labs**

| ***Lab*** | ***MSU*** | ***Pure SSU[1]*** | ***Pure Seed in MSU*** | ***Inert in MSU*** | ***Free Inert[2]*** | ***Time saved by method 3 (min)*** | ***Total Pure Seed by AOSA*** | ***Total Pure Seed by method 3*** |
|---|---|---|---|---|---|---|---|---|
| ***1*** | 4.36* | 94.11 | 3.25 | 1.07 | 1.52 | 49 | 97.36 | 98.48 |
| ***2*** | 3.64* | 95.01 | 2.57 | 1.14 | 1.36 | 51 | 97.58 | 98.64 |
| ***3*** | 4.13 | 93.92 | 2.91 | 1.22 | 1.95 | 54 | 96.83 | 98.05 |
| ***4*** | 1.99* | 96.11 | 1.28 | 0.69 | 1.90 | 35 | 97.39 | 98.10 |
| ***5*** | 1.99* | 97.22 | 1.35 | 0.62 | 0.78 | 29 | 98.57 | 99.22 |
| ***6*** | 3.73 | 94.88 | 2.72 | 1.01 | 1.39 | 40 | 97.60 | 98.61 |
| *7* | 3.79 | 94.26 | 2.89 | 0.90 | 1.95 | 52 | 97.15 | 98.05 |
| ***Grand Mean*** | ***3.38*** | ***95.07*** | ***2.42*** | ***0.95*** | ***1.55*** | ***44*** | ***97.50*** | ***98.45*** |
| | | | | | | | ***0.30*** | ***0.18*** |

***** Total MSU% (column 2) before dismembering does not match the sum of pure seed and inert matter from MSU (columns 4 +5) for some labs. Variation may have resulted from weighing each component independently (i.e., total MSU, pure seed in MSU, and inert in MSU). Data were recorded as received from the labs.

[1] *Single Seed Units not attached to any other seed structure.* [2] *Not from multiple seed units.*

**Table 3. Mean Percentage of Sample Components Including Multiple Seed Units (MSU),** Pure Seed and Inert Matter in 20 TF Samples Tested at 7 Labs

| *Lab* | *MSU* | *Pure SSU*[1] | *Pure Seed in MSU* | *Inert in MSU* | *Free Inert*[2] | *Time saved by method 3 (min)* | *Total Pure Seed by AOSA* | *Total Pure Seed by method 3* |
|---|---|---|---|---|---|---|---|---|
| ***1*** | 1.91* | 96.40 | 1.28 | 0.76 | 1.69 | 19 | 97.68 | 98.31 |
| ***2*** | 1.93 | 96.35 | 1.28 | 0.65 | 1.72 | 29 | 97.62 | 98.28 |
| ***3*** | 1.66 | 96.13 | 1.24 | 0.42 | 2.31 | 32 | 97.37 | 97.79 |
| ***4*** | 1.98* | 96.44 | 1.49 | 0.50 | 1.57 | 29 | 97.93 | 98.43 |
| ***5*** | 1.70* | 95.95 | 1.21 | 0.45 | 2.35 | 56 | 97.16 | 97.65 |
| ***6*** | 1.91* | 96.08 | 1.39 | 0.46 | 2.01 | 109 | 97.48 | 97.99 |
| *7* | 1.98* | 96.11 | 1.41 | 0.56 | 1.91 | 27 | 97.53 | 98.09 |
| ***Grand Mean*** | ***1.87*** | ***96.21*** | ***1.33*** | ***0.54*** | ***1.94*** | ***43*** | ***97.54*** | ***98.08*** |
| ***Variance*** | | | | | | | ***0.06*** | ***0.08*** |

* Total MSU% (column 2) before dismembering does not match the sum of pure seed and inert matter from MSU (columns 4 +5) for some labs. Variation may have resulted from weighing each component independently (i.e., total MSU, pure seed in MSU, and inert in MSU). Data were recorded as received from the labs.

[1] *Single Seed Units not attached to any other seed structure.* [2] *Not from multiple seed units.*

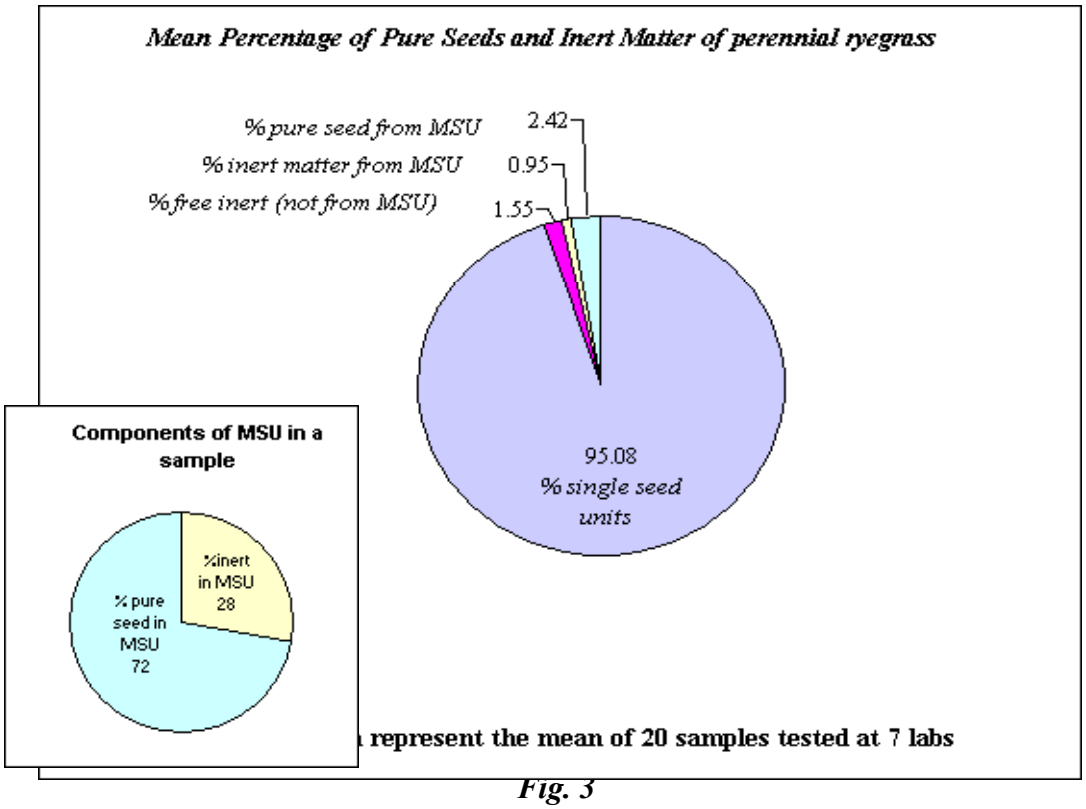


***Fig. 3***

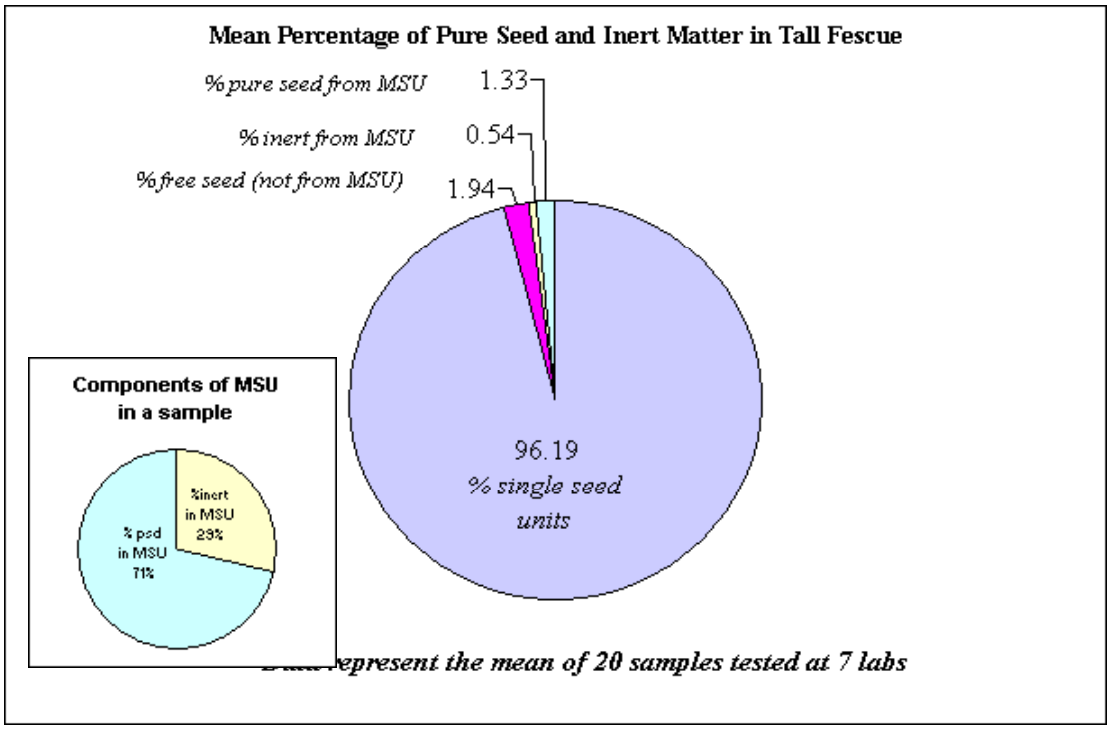


***Fig. 4***

**Feasibility of adopting method 3 in purity testing of PRG and TF**

Data from the referee study and the OSU study were used to compare AOSA method and method 3. In general, Figures 5 and 6 indicated that method 3 provided comparable results to those of AOSA results in the pure seed percentage of both PRG and TF. According to the two studies, applying method 3 increased the pure seed percentage by an average of 0.87-0.95% in PRG and by 0.42-0.54% in TF (Tables 1, 2, & 3). The t-test statistical analysis indicated no significant difference between percentage of pure seed by AOSA method and method 3 in both crops.

The referee study results also showed that the average time saved by using method 3 over AOSA method is 44 minute per sample in PRG, and 43 minute in TF (Tables 2 & 3). Similar results were observed in OSU study (Table 1). However, the time saving may vary due to differences in the methods of separating and breaking the MSU and the speed of seed analysts. Method 3 dramatically increased purity testing speed, even much more than the factor method of AOSA.

The physical separation of MSU into pure seed and inert required by the current AOSA method can also increase variability of purity results among laboratories and even between analysts within the same lab. Method 3 considers multiple florets as part of pure seed fraction; consequently physical separation and other related problems with the current AOSA and factor

methods could be avoided. This is reflected by the increased variance among labs using AOSA method (0.30) over using method 3 (0.18) in PRG (Table 2).

It is worthy to note that Jensen in 1984 had recommended leaving the sterile structures intact and reporting the percentage of MSU for ryegrass. ISTA adopted this recommendation, but the American seed industry had reservation about this method at that time. In 2001, ISTA went a further step by not requiring reporting the MSU percentage in purity testing unless the customer requests this information. This study re-confirmed the feasibility of adopting method 3 (i.e., similar to ISTA method) in dealing with MSU in PRG and TF.


## ACKNOWLEGEMENTS

The Oregon State University Seed Laboratory would like to offer special thanks to the Oregon Seed Council, and the Tall Fescue Commission for supporting these studies. Also, we would like to thank all the seed labs that participated in the national referee study, Oregon seed analysts for their input in these studies, and to Sherry Hanning, the OSU purity supervisor and the OSU purity analysts for the assistance throughout the studies.


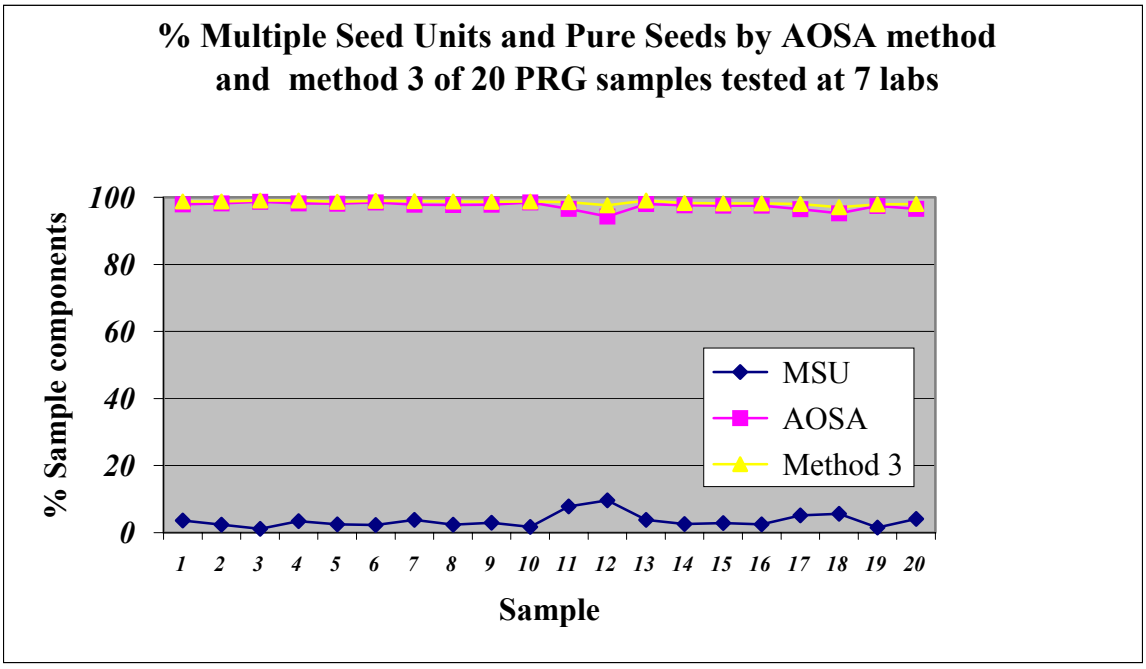


*Fig. 5*

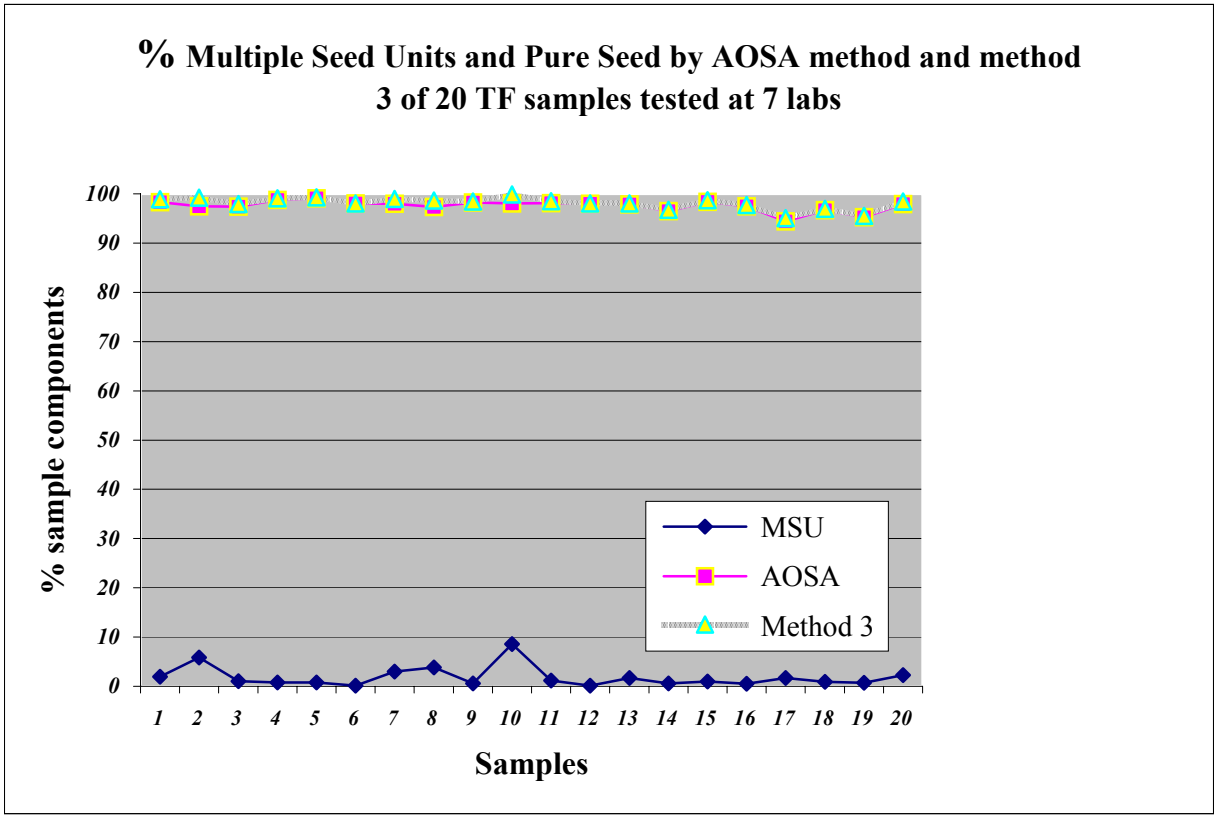


***Fig. 6***

## SUMMARY AND CONCLUSIONS

- Method 3 (i.e., similar to ISTA method) provided comparable % pure seed results to AOSA method in both TF and PRG.
- Using method 3 achieved higher consistency purity results among laboratories in PRG and approximately the same consistency results in TF.
- The average time saved by using method 3 over AOSA method depends on the MSU content in each sample and on the analyst performing the test. In general, the average saving time in these studies was 41 min. in TF and 44 min. in PRG.
- As the % MSU increased in a sample, the difference in % pure seed measured by AOSA method and method 3 increased for PRG and TF.
- The percentage of pure seed in the MSU was 72% for PRG (i.e., constant factor 0.72) and 71% for TF (i.e., constant factor 0.71).
- 80% of TF the 100 samples used in study (1) had less than 2% MSU, and 95% of the samples had less than 4%.
- 62% of PRG the samples used in study (1) had less than 4% MSU, and 83% had less than 6%MSU.
- The studies supported the feasibility of adopting method 3 for purity analysis of PRG and TF.

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**Purity Analysis Comparative Study of Two Methods**

**Dealing with Multiple Seed Units in Ryegrass and Tall Fescue**

Sabry Elias, Sharon Davidson and Deborah Meyer

This study was conducted to compare the AOSA method of manually dismembering multiple seed units (MSU) in purity testing of *Lolium* spp. and *Festuca arundinacea* to an ISTA-like method where the MSU is left intact in the pure seed fraction as long as there is at least a floret with some degree of endosperm development.

## Objective

Measure the effects of leaving the MSU intact within the pure seed fraction (Method 1) and the AOSA method (Method 2) on pure seeds, inert matter percentage and time to complete the purity tests of annual, perennial, and intermediate ryegrass; and tall fescue.

## Materials and Methods

A total of 15 laboratories conducted purity tests on 27 annual ryegrass, 45 perennial ryegrass, 9 intermediate ryegrass, and 71 tall fescue seed samples. Pure seed and inert matter percentage were determined for each sample (Table 1). The tests were carried out on commercial samples available in the participating labs as part of their routine testing operations.

Each seed sample was tested twice using two methods: Method 1: leaving the MSU intact and including them in the pure seed fraction providing they had at least one seed with some degree of endosperm development, and Method 2: following the AOSA method, which requires separating the MSU into pure seed and inert matter.

The pure seed and the inert matter percentages and the time to complete each test were recorded. The results of the two methods were compared for each species.

## Results and Discussion

The pure seed fraction as measured by method 1 (i.e., similar to ISTA method) were higher by 0.13, 0.68, 0.22, and 0.37 percent compared to the AOSA method for annual, perennial, intermediate ryegrass, and tall fescue, respectively. The inert matter content of method 2 ( AOSA) was higher by 0.6, 0.56, 0.03, and 0.36 percent than method 1 for annual, perennial, intermediate ryegrass, and tall fescue, respectively. These data represented the mean of all samples tested in each species, averaged over all participating labs (Table 1). The higher inert matter reported by Method 2 (AOSA) resulted from dismembering the MSU creating artificial inert matter, which does not reflect the actual purity of a seed sample.

The time to complete the purity tests using Method 1 (similar to ISTA) was 18-42% faster than the AOSA method depending on the species and amount of MSU in the sample. Method 1 was more efficient than the AOSA method, especially for perennial ryegrass (Table 1). As the percentage of MSU in a sample increases, the time required to break and separate MSU into pure seed and inert matter also increases (data not shown).

Meyer, D. reported similar results when she compared the above two methods for *Festuca pratensis* Hudson, and *Lolium* X *hybridium* Haussknecht (Meyer, D. L. 2000). Elias et al.

reached similar conclusions when comparing the same two methods for *Lolium perenne* and *Festuca arundinacea.*

## Conclusions

The multiple seed units are regarded and handled as seed units in typical planting practices and commercial channels. The results suggest that including the MSU in the pure seed fraction is more realistic than manually breaking and separating them. Adopting Method 1 will avoid creating artificial inert matter and will provide comparable pure seed and inert matter results to those obtained using the AOSA method. It would also contribute to greater objectivity and repeatability of results among and within labs. Method 1 (similar to ISTA) will also save time in purity testing and will harmonize the pure seed definition for *Lolium spp a*nd *Festuca arundinacea* with ISTA Rules.

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Table 1. Mean percentage of pure seed, inert matter and purity testing time using two methods in dealing with multiple seed units in annual, perennial, intermediate ryegrass, and tall fescue.

| KIND | NO. OF SAMPLES TESTED | NO. OF LABS ** | METHOD 1† | | | AOSA METHOD‡ | | |
|---|---|---|---|---|---|---|---|---|
| | | | % Pure | % Inert | Time (min) | % Pure | % Inert | Time (min) |
| Annual Ryegrass* | 27 | 10 | 98.81 | 0.91 | 18 | 98.68 | 0.97 | 22 |
| Perennial Ryegrass | 45 | 12 | 98.55 | 1.34 | 19 | 97.87 | 1.90 | 33 |
| Intermediate Ryegrass | 9 | 3 | 98.88 | 0.56 | 6 | 98.66 | 0.53 | 8 |
| Tall Fescue* | 71 | 12 | 97.55 | 1.16 | 26 | 97.17 | 1.52 | 32 |

† Method 1: leaving the MSU intact and including them in the pure seed fraction (i.e., similar to ISTA method), providing they had at least one seed with some degree of endosperm development.‡ AOSA method: requires separating the MSU into pure seed and inert matter.* Data was excluded when no comparison was available between method 1 and AOSA Method.** A total of 15 laboratories provided data in the study.

# Comparison of Two Methods of Purity Testing for *Festuca pratensis* Hudson and *Lolium* x *hybridum* Haussknecht

Deborah J. Lionakis Meyer

## ABSTRACT

**Comparisons among the AOSA purity testing methods for meadow fescue (*Festuca pratensis* Hudson) and intermediate ryegrass (*Lolium* x *hybridum* Haussknecht), and a new method in which only the large sterile structures are removed were made by the AOSA Purity Subcommittee. Pure seed percentage, number and types of seed unit attachments, amount of inert material recovered from the attachments and the time required for inert recovery were examined. The new method produced nearly identical purity results to the AOSA method for both species with a considerable time savings.**

## INTRODUCTION

According to the AOSA Rules for Testing Seeds (AOSA, 1998), seed units for meadow fescue and intermediate ryegrass include single florets and caryopses. By definition, analysts are required to detect and remove all structures attached to the rachilla of the fertile floret and all structures attached to the base of the fertile floret. This is a laborious and time consuming process. Comparisons among the AOSA and ISTA (ISTA, 1996) purity testing methods for annual ryegrass (*Lolium multiflorum* Lamarck), perennial ryegrass (*L. perenne* Lamarck) and tall fescue (*Festuca arundinacea* Schreber), and a third method in which only the large sterile structures are removed were made by the AOSA Purity Subcommittee (Meyer 1997). Based on this study AOSA adopted a new seed unit definition for annual ryegrass, perennial ryegrass and tall fescue in which certain small sterile structures are allowed to remain attached to the rachilla as part of the pure seed unit (AOSA 1998).

## METHOD

Sixteen seed analysts from eleven laboratories with experience in testing meadow fescue and intermediate ryegrass, were provided 5 g sub-samples of six commercial seed lots of meadow fescue and 8 g sub-samples of eight commercial seed lots of intermediate ryegrass. Each sub-sample was separated into the following four categories: (1) single fertile florets without attached structures (Fig. 1, A) and free caryopses; (2) fertile florets with any structures attached to the rachilla or basally attached glume (Fig. 1; B, C, D & E); (3) extraneous inert matter; and (4) seed units of species other than the kind being tested. The time (minutes) required to make the separation and the weight (grams) of each component was recorded. Florets from the second category were further segregated into two groups: Type-A – fertile florets with

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FIGURE 1. Types of fertile florets: A - single fertile floret; B and C - fertile floret with attached structure not extending to the tip of the fertile floret; D - fertile floret with attached structure equal to or longer than tip of fertile floret; E - fertile floret with basally attached glume.

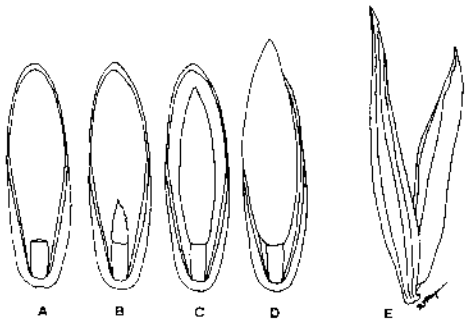


TABLE 1. Mean percent pure seed for six samples of Meadow Fescue and eight samples of Intermediate Ryegrass as determined using two purity methods in eleven laboratories, and mean differences of pure seed percentage between the AOSA Method and the New Method.

| Crop | Percent Pure Seed | | Mean Difference Between Methods |
|---|---|---|---|
| | AOSA Method | New Method | |
| | ---------- | ---------- | % ---------- |
| Meadow Fescue | 98.77 | 98.81 | -0.04 |
| Intermediate Ryegrass | 99.42 | 99.43 | -0.01 |

TABLE 2. Coefficients of variation of mean pure seed percentage when determined by two purity methods.

| Crop | AOSA Method | New Method |
|---|---|---|
| | ---------- | % ---------- |
| Meadow Fescue | 0.86 | 0.86 |
| Intermediate Ryegrass | 0.24 | 0.23 |

TABLE 3. Mean time (minutes) to recover attached inert material, percent inert material recovered, and relationship between the number of Type-A and Type-B florets detected and time required to recover attached inert material using AOSA Method.

| Crop | TYPE-A FLORETS | | | TYPE-B FLORETS | | |
|---|---|---|---|---|---|---|
| | Mean Time (M) | Mean Inert (%) | Correlation Number/Time (r-value) | Mean Time (M) | Mean Inert (%) | Correlation Number/Time (r-value) |
| Meadow Fescue | 4 | 0.19 | 0.61* | 11 | 0.04 | 0.90* |
| Intermediate Ryegrass | 2 | 0.05 | 0.84* | 3 | 0.01 | 0.73* |

*Significant at the 5% level

attached sterile structures equal to or longer than the fertile floret (Fig. 1, D), fertile florets with basally attached glumes (Fig. 1, E), fertile florets attached to other fertile floret(s); and Type-B – fertile florets with attached sterile structures not extending to the tip of the fertile floret (Fig. 1, B & C), excluding the awn if applicable. Weights of the Type-A and Type-B florets were recorded. The weight of inert material detached from the fertile florets in the Type-A group along with the time required to remove the structures was also recorded. The same procedure was followed for the Type-B group. Data were combined to provide results of two different test methods: AOSA Method = all attached structures on Type-A and Type-B florets were removed and classified as inert material or fertile florets; and New Method = sterile florets and glumes attached to Type-A florets were removed and classified as inert material, attached fertile florets were separated and Type-B florets were kept intact. Data from each species were analyzed separately, the six meadow fescue subsamples and eight intermediate ryegrass sub-samples were regarded as six and eight replicates, respectively, tested by each laboratory. Information about equipment used to examine the florets was recorded.

## RESULTS

Complete data sets were received from all laboratories except Lab 8, which completed only samples 1–5 for meadow fescue and 1–4 for intermediate ryegrass. Mean percent pure seed for each method and crop type, and mean percent difference between the two methods are shown in Table 1. Results from both methods were nearly identical for both species and not statistically different (student-t, $p = 0.05$). The coefficients of variation (cv) showed little difference between the two methods (Table 2). Pure seed percentages obtained by the New Method were slightly less variable for intermediate ryegrass, while variation among those obtained for meadow fescue were the same regardless of the method used.

Mean time (minutes) to recover inert material from Type-A and Type-B florets and mean percent inert material recovered from each type of floret per crop are shown in Table 3. The number of Type-A and Type-B florets detected showed significant correlation with the amount of time required to recover attached inert material. More time was spent recovering inert material from Type-B florets than Type-A for both species tested. The type of equipment used to examine the florets and the number of Type-B florets detected for meadow fescue and intermediate ryegrass are shown in Figures 2 and 3, respectively. Numbers of Type-B florets detected varied considerably among laboratories, with high and low detection levels distributed among equipment types used (i.e., <2X magnifying lens, 5–7X handlens, or microscope). Random variation may account for some differences, however three laboratories were consistently low in detection of Type-B florets for both species. Mean times to complete each portion of a purity test following the AOSA Method for each species are shown in Figure 4. Mean time required to recover attached inert material from Type-B florets represents the mean time saved using the New Method.

## CONCLUSIONS

For the samples tested, the New Method produced nearly identical results to the AOSA Method for both species with a considerable time savings. Detection of Type-B florets may have been a problem for some laboratories regardless of the equipment type used, however this was not reflected in the amount of inert material recovered from Type-B florets or the percentage of pure seed.

## ACKNOWLEDGMENTS

The author would like to thank the following seed laboratories for their participation: Agri Seed Testing (Sharon Davidson), Agri Seed Testing (Jane Penrose), California State Seed Laboratory (Jim Effenberger), Georgia State Seed Laboratory, Atlanta ( Aida Galarza, Lonnita André, D. Hembree, A. Tye, and Carol Perry), Growmark (Stewart Oliver), Maryland State Seed Laboratory (Jennifer Miller), Pennsylvania State Seed Laboratory (William Cook), Utah State Seed Laboratory (Stan Akagi), Washington State Seed Laboratory (Victor Shaul), Ransom Seed Lab (Sue Alvarez), Wyoming State Seed Lab (Billie Lundberg). Thank you also to Stewart Oliver, Aida Galarza, and Victor Shaul for providing the seed for the project.

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FIGURE 2. Number of Type-B Meadow Fescue florets found in six samples by eleven laboratories, and type of equipment used.

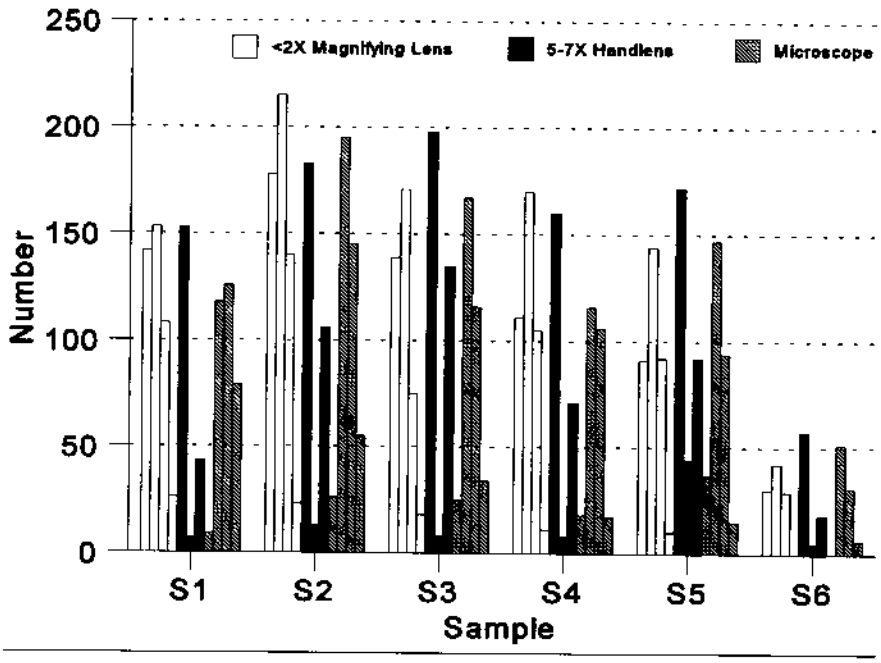

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FIGURE 3. Number of Type-B Intermediate Ryegrass florets found in eight samples by eleven laboratories, and type of equipment used.

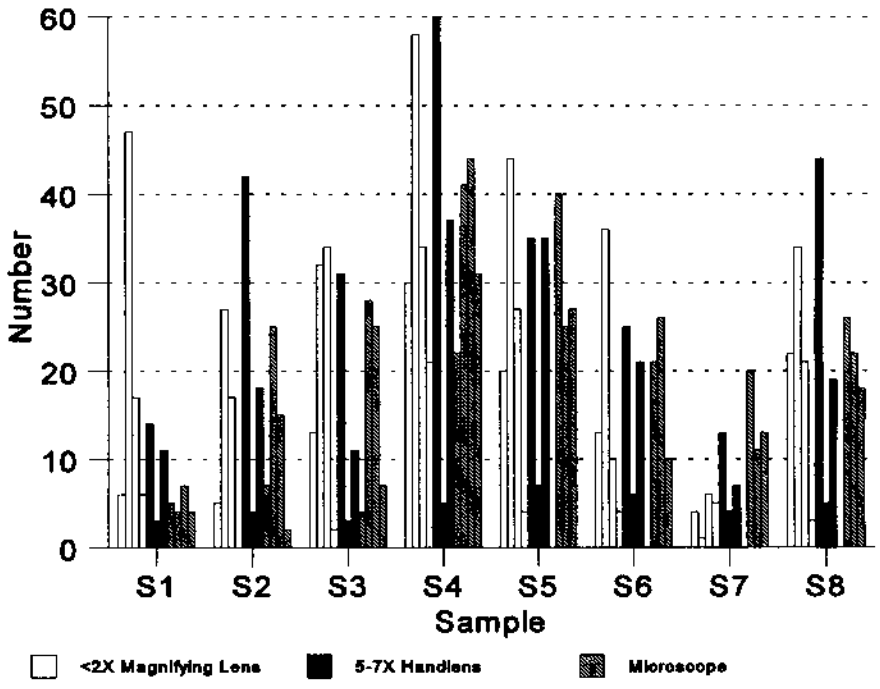
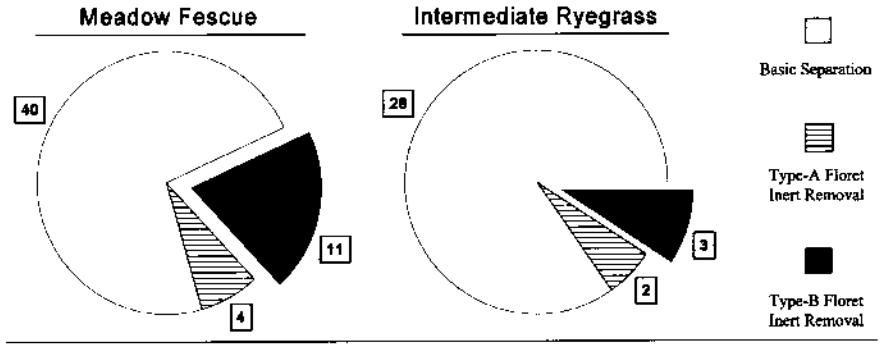



FIGURE 4. Mean time (minutes, in boxes) required to complete each separation for AOSA Method. Mean time required to remove attached inert material from Type-B florets represents mean time saved using New Method.



**OREGON STATE UNIVERSITY SEED LABORATORY**

# Germination of Perennial Ryegrass Seeds with Caryopses Less than one-third the Length of the Palea

*Sabry Elias and Adriel Garay*

**ABSTRACT**

It has been long debated among seed analysts whether to define caryopses with some degree of endosperm development as pure seed or inert matter. This study was conducted to evaluate the germinability of perennial ryegrass seed (*Lolium perenne*) that contain caryopses less than one-third of the length of the palea and compare them with seed that contain caryopses at least one-third of the length of the palea. Random samples from forty seed lots representing two cultivars harvested in 1999, 2000, and 2001 were examined. Seeds with caryopses having some degree of endosperm development were separated from each sample and germinated following the AOSA Rules for Testing Seeds. One hundred seeds with caryopses one-third the length of the palea or more from each sample were also germinated as control checks. The germination of seeds with caryopses less than one-third of the length of the palea was 0.33%, compared to 92% for seeds with more than one-third of the length of the palea. The results suggested that seeds with caryopses less than one-third of the length of the palea should not be classified as pure seed. Including such seed in the pure seed fraction of a sample would result in decreasing its germination percentage.

## INTRODUCTION

According to the Rules for Testing Seeds of the Association of Official Seed Analysts, a ryegrass floret with any visual degree of endosperm development is classified as pure seed. On the other hand, the Seed Testing Rules of the International Seed Testing Association specify that ryegrass seeds with caryopses less than one-third the length of palea should not be considered as pure seed. Evidently there is a difference between these two rules.

This study was conducted to examine the germinability of perennial ryegrass (*Lolium perenne*) seeds with caryopses less than one-third the length of the palea. The hypothesis is that if such seeds do not germinate, they should be classified as empty seed and hence as a part of the inert matter, not the pure seed portion.

Similar studies have been performed previously in other species. In 1997, a referee study was conducted by Jane Penrose of Agri-Seed Testing Laboratory to measure the germinability of tall fescue (*Festuca arundinacea*) seeds with caryopses less than one-third the length of the palea. The results showed that only one seed out of 2500 seeds (0.04%) with caryopses less than one-third the length of the palea produced a normal seedling. Earlier study by Everson (1954) indicated that the germination percentage of quackgrass (*Agropyron repens*) seeds with caryopses less than one-third the length of the palea was 0%. His results were consistent across three locations for two years.

## MATERIALS AND METHODS

Seed samples from 40 different seed lots, representing two randomly chosen perennial ryegrass varieties 'Citation III' and 'Sonata', from 1999, 2000 and 2001 crops were used in the study. The sample size used to find the seeds with caryopses less than one-third the length of the palea ranged from 100-150 grams depending on the availability of seed. A diaphanoscope was used to visually separate such seeds from each sample. Tables 1 and 2 show the number of seeds with caryopses less than one-third the length of the palea found in each sample, which ranged from 0 to 60 seeds. All seeds from each sample were planted separately in a plastic box following the AOSA Rules. One hundred seeds with caryopses more than one-third the length of the palea were also planted side by side as a control check for each of the 40 samples. All seeds were planted on August 6, 2002 without chilling as the seeds were one year or older with no apparent degree of dormancy. The germination percentage was recorded after 14 days.

## RESULTS AND DISCUSSION

The mean germination of seeds with more than one-third the length of the palea (i.e., the control check) of the 40 samples was 92% (Tables 1 & 2; and Figure 1). In contrast, out of the 303 seeds with caryopses less than one-third the length of the palea, separated from the 40 seed samples, only one seeds germinated (0.33%). No root or shoot growth was observed in the rest of the seed. At the end of the germination period, no mold growth was observed and few decayed and abnormal seeds were found. The fact that only one seed germinated and produced a normal seedling under optimum germination conditions of moisture, temperature, light, and substrata free from any soil microorganisms, indicates that the seeds with caryopses less than one-third the length of the palea have rudimentary embryos and limited amount of storage substances. In most cases, this is insufficient for the development of new plant. The only seed with some visible degree of endosperm development that germinated, produced a less vigorous seedling than those of the control checks (Fig. 1). As expected, samples contained different numbers of seeds with a caryopses that was less than one-third the length of the palea depending on the growing conditions and the level of cleaning. If the air-flow settings in cleaning equipment are below optimum, more seeds with a visible degree of endosperm or with caryopses less that one-third of palea length are expected in the pure seed portion.

The results from this study are consistent with the results reported by Jane Penrose (1997) and Everson (1954) studies. The fact that seeds with caryopses less than one-third of the length of the palea do not germinate implies that the inclusion of such seeds as part of the pure seed fraction can not be justified. Furthermore, its inclusion in the germination test would arbitrarily lower the germination percentage.

## CONCLUSIONS

These results provide a realistic basis for a rule proposal to classify seeds with caryopses less than one-third the length of the palea as inert matter. Furthermore, seeds with a caryopses that is one-third the length of the palea are much easier to detect than seeds with some degree of visible endosperm development. This would provide an objective and easier standard to make consistent decisions if the floret is a seed or inert. Consequently, it would reduce variability among laboratories resulting from subjectivity in classifying seeds with any

degree of visible endosperm development. It would also harmonize AOSA and ISTA Rules in regard to this standard.

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***Table 1. Germination percentage of seeds with caryopses less than one-third the length of the palea and normal seeds with caryopses more than one-third the length of the palea of two perennial ryegrass varieties from 2001 crop.***

| No. | Variety | Seed with caryopses less than 1/3 the length of the palea | | % germination of seeds with caryopses more than 1/3 the length of the palea |
|---|---|---|---|---|
| | | No. in sample | No. Germinated | |
| 1 | Citation III | 3 | 0 | 95 |
| 2 | Citation III | 4 | 0 | 88 |
| 3 | Citation III | 2 | 0 | 90 |
| 4 | Citation III | 2 | 0 | 96 |
| 5 | Citation III | 2 | 0 | 95 |
| 6 | Citation III | 5 | 0 | 88 |
| 7 | Citation III | 1 | 0 | 96 |
| 8 | Citation III | 1 | 0 | 94 |
| 9 | Citation III | 3 | 0 | 95 |
| 10 | Citation III | 6 | 0 | 95 |
| 11 | Sonata | 2 | 0 | 93 |
| 12 | Sonata | 2 | 0 | 86 |
| 13 | Sonata | 5 | 0 | 88 |
| 14 | Sonata | 4 | 0 | 92 |
| 15 | Sonata | 29 | 0 | 96 |
| 16 | Sonata | 0 | 0 | 93 |
| 17 | Sonata | 3 | 0 | 95 |
| 18 | Sonata | 1 | 0 | 86 |
| 19 | Sonata | 13 | 0 | 94 |
| 20 | Sonata | 2 | 0 | 87 |
| | | ***Total: 90*** | ***Total: 0*** | ***Mean: 92.1*** |

Table 2. Germination percentage of seeds with caryopses less than one-third the length of the palea and normal seeds with caryopses more than one-third the length of the palea of two perennial ryegrass varieties from 1999 and 2000 crops.

| No. | Variety | Seed with caryopses less than 1/3 the length of the palea | | % germination of seeds with caryopses more than 1/3 the length of the palea |
|---|---|---|---|---|
| | | No. in sample | No. Germinated | |
| 1 | Citation III | 3 | 0 | 94 |
| 2 | Citation III | 27 | 0 | 92 |
| 3 | Citation III | 12 | 0 | 90 |
| 4 | Citation III | 0 | 0 | 97 |
| 5 | Citation III | 0 | 0 | 97 |
| 6 | Citation III | 2 | 0 | 92 |
| 7 | Citation III | 2 | 0 | 86 |
| 8 | Citation III | 58 | 0 | 73 |
| 9 | Citation III | 20 | 0 | 87 |
| 10 | Citation III | 60 | 1 | 94 |
| 11 | Sonata | 1 | 0 | 96 |
| 12 | Sonata | 0 | 0 | 91 |
| 13 | Sonata | 7 | 0 | 93 |
| 14 | Sonata | 1 | 0 | 95 |
| 15 | Sonata | 1 | 0 | 96 |
| 16 | Sonata | 10 | 0 | 94 |
| 17 | Sonata | 6 | 0 | 98 |
| 18 | Sonata | 2 | 0 | 88 |
| 19 | Sonata | 1 | 0 | 94 |
| 20 | Sonata | 0 | 0 | 88 |
| | | ***Total: 213*** | ***Total: 1*** | ***Mean: 91.75*** |

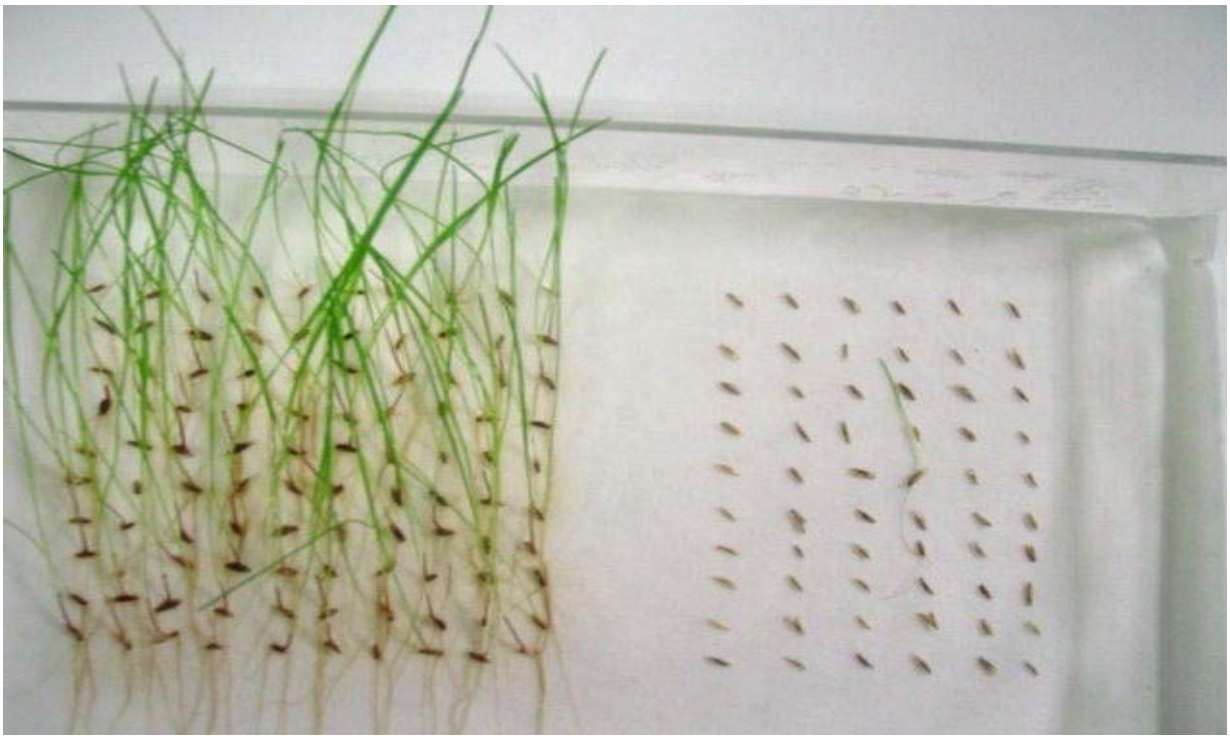

***Fig. 1. Out of the 303 seeds with caryopses less than one-third the length of the palea, separated from 40 seed samples, only one seed germinated (0.33%).***

**Effect of single florets with caryopsis less than one-third the length of the palea on pure seed and inert matter components of ryegrass and tall fescue**

**Sabry Elias, Sherry Hanning, and Adriel Garay**

## ***Introduction*:**

The pure seed definition for *Lolium* spp. *and Festuca arundinacea* in the Rules for Testing Seeds of the AOSA (2001) states that florets with some degree of endosperm development should be classified as pure seed. On the other hand, the ISTA Rules states that a floret should have at least a caryopsis 1/3 the length of the palea (Fig. 1) to be considered pure seed. A study conducted by Jane Penrose (AgriSeed Testing) in tall fescue, and Sabry Elias and Adriel Garay (Oregon State University) in perennial ryegrass, demonstrated that caryopses less than 1/3 the length of the palea do not have a capacity to germinate. These results suggest that those florets that have caryopses less than 1/3 the length of the palea should be considered inert. However, there is no available data to quantify the effect of classifying seeds with caryopses less than 1/3 the length of the palea as inert matter on pure seed and inert matter components in purity testing.

Therefore, this study was designed to measure the contribution of florets with caryopses less than one-third the length of the palea to the amount of pure seed using the AOSA rules or to the inert matter under the proposed rule. In the proposed rule, florets with caryopses less that 1/3 the length of the palea is classified as inert matter.

This study also, compared the change in pure seed, and inert matter components as well as the time needed to complete purity tests on ryegrass and tall fescue following the current AOSA pure seed definition and the proposed definition, which states that multiple seed units that include at least one floret with caryopsis 1/3 the length of the palea are left intact and included in the pure seed fraction, and that florets with caryopses less than 1/3 the length of the palea are classified as inert matter.

## ***Objectives*:**

The objectives of this study were to:

1. Measure the contribution of single florets with caryopses less than 1/3 the length of the palea (i.e., have some degree of endosperm development as the current AOSA states) to pure seed and inert matter of purity testing results of ryegrass and tall fescue using both the current AOSA method and the proposed method.
2. Compare the change in pure seed, and inert matter components of ryegrass and tall fescue following the current AOSA pure seed definition and the proposed definition.
3. Compare the time required to complete purity tests using the proposed pure seed definition method and the current AOSA method for ryegrass and tall fescue.

## ***Materials and methods***:

Twenty perennial ryegrass, 20 tall fescue samples, and 5 annual ryegrass (all from 2002 crop) were used in the study. The samples were randomly selected as they were entered for testing at the OSU Seed Laboratory. The following tests were conducted on each sample:

1. Purity test according to the proposed pure seed definition, which leaves multiple seed units that include at least one floret with caryopsis 1/3 the length of the palea intact and include them in the pure seed fraction and classify any florets with caryopses less that 1/3 the length of the palea as inert matter.
2. Single florets with caryopses less than 1/3 the length of the palea in each sample were separated, counted and weighed in each sample. The percentage of such seeds in each sample was calculated and its effect in increasing the total inert matter (in the proposed method) and decreasing the pure seed component (in the AOSA method) was recorded.
3. All sample components were added back together to test the sample according to the seed unit definition number 21 of the AOSA Rules for Testing Seeds (2001).
4. The time spent in conducting purity tests using the current AOSA method and the proposed method for each sample was recorded for comparison.

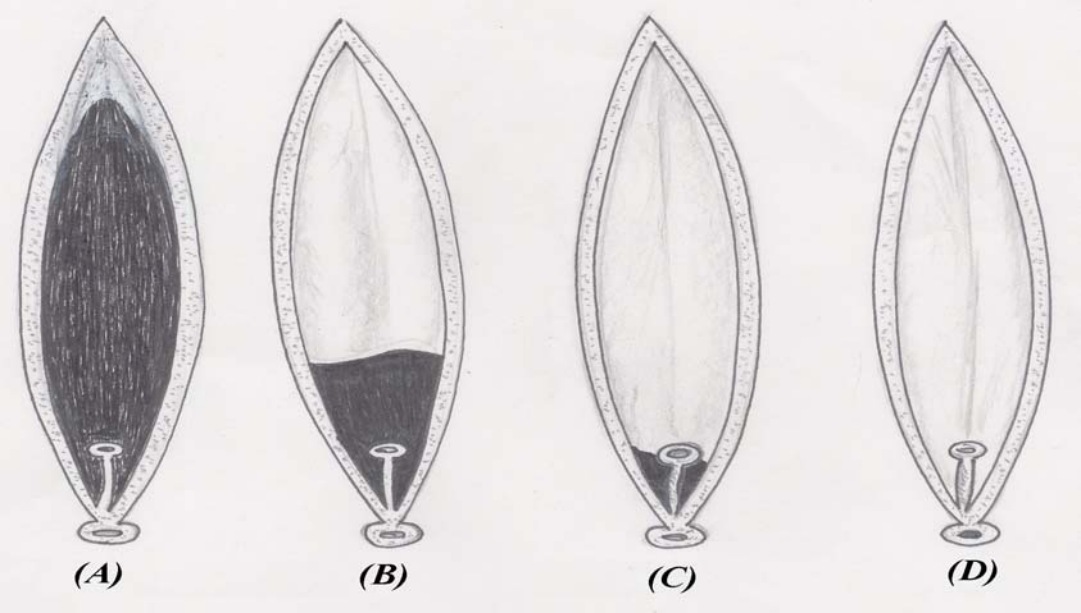


Fig. 1. Stylized diagram of (A) Fully developed caryopsis, (B) Caryopsis 1/3 the length of the palea ,(C) Caryopsis with some degree of endosperm, and (D) Empty floret with no caryopsis (seed) inside.

***Results and discussion***:

### *I. The effect of florets with caryopses less than 1/3 the length of the palea on pure seed and inert matter component of ryegrass and tall fescue:*

According to the proposed pure seed definition rule single florets with caryopses less than one-third the length of the palea should be classified as inert matter. The results of this study showed that this will slightly increase the inert matter portion in purity testing with an average of 0.01, and 0.09% for perennial ryegrass and tall fescue, respectively (Tables 1, and 2). In annual ryegrass, the amount of increase in the inert matter portion was found to be even smaller, only

0.002% (data not shown). Similarly, if florets with caryopses less than one-third the length of the palea were re-classified from pure seed (as it is currently classified in the AOSA Rules) to inert matter, the decrease in the amount of pure seed portion will be generally minimal in annual and perennial ryegrass as well as in tall fescue (i.e., 0.002, 0.01, and 0.09%, respectively). In general, the effect of florets with caryopses less than one-third the length of the palea, is proportional to the number of such florets in each sample (Tables 1 and 2).

Considering the fact that florets with caryopses less than one-third the length of the palea do not have capacity to germinate (Elias and Garay, 2002; Everson, 1954; Penrose, 1997), classifying them as inert matter would be appropriate. Such classification will also harmonize AOSA Rules with ISTA, which contributes to facilitating the global seed trade of these crops. In addition, this classification is more objective, and less cumbersome from the seed analysis point of view.

Table 1. Comparison of pure seed and inert matter components of Perennial ryegrass as tested by two methods

| | *Proposed Method** | | | *AOSA* | | | *Differences between the two methods* | | |
|---|---|---|---|---|---|---|---|---|---|
| sample | % pure seed | % inert matter | time/min | % pure seed | % inert matter | time/min | % increase in purity using the proposed method | % increase in inert using the AOSA method | % seeds less than 1/3 length of palea |
| 1 | 99.557 | 0.4432 | 13 | 98.713 | 1.2874 | 45 | 0.8442 | 0.8442 | 0.0326 |
| 2 | 99.543 | 0.1961 | 15 | 99.221 | 0.6988 | 35 | 0.3218 | 0.5027 | 0.0152 |
| 3 | 99.089 | 0.9108 | 15 | 98.215 | 1.7153 | 40 | 0.8741 | 0.8045 | 0.0155 |
| 4 | 99.439 | 0.5614 | 14 | 98.858 | 1.1418 | 37 | 0.5803 | 0.5803 | 0.0151 |
| 5 | 99.663 | 0.3373 | 8 | 99.597 | 0.4032 | 22 | 0.0659 | 0.0659 | 0.0155 |
| 6 | 97.990 | 1.8063 | 26 | 96.832 | 2.5592 | 52 | 1.1573 | 0.7529 | 0.0154 |
| 7 | 98.209 | 1.7418 | 22 | 96.820 | 2.7406 | 50 | 1.3885 | 0.9989 | 0.0153 |
| 8 | 98.280 | 1.1919 | 23 | 97.188 | 1.9595 | 45 | 1.0916 | 0.7676 | 0.0154 |
| 9 | 98.481 | 1.1007 | 15 | 96.182 | 3.0374 | 52 | 2.2991 | 1.9367 | 0.0153 |
| 10 | 98.964 | 0.7948 | 12 | 98.214 | 1.3465 | 27 | 0.7504 | 0.5517 | 0.0154 |
| 11 | 98.704 | 0.8892 | 23 | 97.425 | 1.9837 | 42 | 1.2786 | 1.0945 | 0.0155 |
| 12 | 97.729 | 1.6950 | 25 | 97.274 | 2.2747 | 37 | 0.4553 | 0.5797 | 0.0153 |
| 13 | 99.289 | 0.7111 | 12 | 98.806 | 1.1936 | 25 | 0.4825 | 0.4825 | 0.0155 |
| 14 | 99.363 | 0.1899 | 14 | 98.972 | 0.3991 | 20 | 0.3914 | 0.2091 | 0.0153 |
| 15 | 99.017 | 0.3162 | 13 | 98.936 | 0.4164 | 18 | 0.0810 | 0.1003 | 0.0154 |
| 16 | 97.754 | 2.2464 | 25 | 96.344 | 3.1874 | 27 | 1.4096 | 0.9410 | 0.0154 |
| 17 | 97.578 | 1.9197 | 19 | 96.593 | 2.6197 | 33 | 0.9851 | 0.7000 | 0.0155 |
| 18 | 97.707 | 2.2573 | 28 | 96.606 | 3.0129 | 43 | 1.1013 | 0.7556 | 0.0151 |
| 19 | 97.873 | 2.1302 | 22 | 97.754 | 2.1997 | 26 | 0.0888 | 0.0695 | 0.0154 |
| 20 | 98.806 | 1.1046 | 23 | 97.891 | 1.7071 | 45 | 0.9151 | 0.6025 | 0.0152 |
| *Mean* | *98.650* | *1.1272* | *18* | *97.822* | *1.794* | *36* | *0.828* | *0.667* | *0.0162* |

*Multiple seed units that include at least one caryopsis with 1/3 the length of the palea are left intact and included in the pure seed fraction. Any floret with caryopses less than 1/3 the length of the palea is classified as inert matter.

II. Changes in pure seed and inert matter components of ryegrass and tall fescue following the current AOSA and the proposed pure seed definition.

The proposed pure seed definition states that multiple seed units that include at least one floret with caryopsis 1/3 the length of the palea are left intact and included in the pure seed fraction. This study measured the average percentage of increase in the pure seed portion using the proposed pure seed definition. The results showed that the average increase in pure seed portion following the new rule was 0.53, and 0.83% compared to the current AOSA rule for tall fescue and perennial ryegrass, respectively (Tables 1 and 2). This increase was found to be only 0.09% in annual ryegrass (Data not shown). Again, the increase in the pure seed portion is proportional to the amount of multiple seed units in each sample (Tables 1 and 2).

Table 2. Comparison of pure seed and inert matter components of Tall fescue as tested by two methods

| | *Proposed Method** | | | *AOSA* | | | *Differences between the two methods* | | |
|---|---|---|---|---|---|---|---|---|---|
| sample | % pure seed | % inert matter | time/min | % pure seed | % inert matter | time/min | % increase in purity using the proposed method | % increase in inert using the AOSA method | % seeds less than 1/3 length of palea |
| 1 | 99.531 | 0.0791 | 6 | 99.419 | 0.0810 | 7 | 0.1119 | 0.0019 | 0.0000 |
| 2 | 98.623 | 0.8966 | 13 | 97.978 | 0.9615 | 14 | 0.6448 | 0.0649 | 0.2098 |
| 3 | 99.369 | 0.1036 | 6 | 98.945 | 0.2282 | 8 | 0.4239 | 0.1247 | 0.0000 |
| 4 | 99.819 | 0.0289 | 6 | 99.660 | 0.0733 | 8 | 0.1582 | 0.0444 | 0.0116 |
| 5 | 98.927 | 0.1996 | 45 | 98.184 | 0.5090 | 50 | 0.7428 | 0.3090 | 0.0653 |
| 6 | 97.861 | 0.8332 | 60 | 96.366 | 1.8450 | 95 | 1.4955 | 1.0116 | 0.0727 |
| 7 | 97.865 | 1.5436 | 45 | 97.309 | 1.6832 | 50 | 0.5566 | 0.1396 | 0.2593 |
| 8 | 98.650 | 0.0531 | 30 | 98.444 | 0.1346 | 40 | 0.2066 | 0.0815 | 0.0114 |
| 9 | 99.418 | 0.2892 | 6 | 99.064 | 0.4348 | 9 | 0.3535 | 0.1456 | 0.0000 |
| 10 | 99.167 | 0.3659 | 21 | 99.009 | 0.3639 | 17 | 0.1582 | -0.0019 | 0.0191 |
| 11 | 96.514 | 2.9035 | 37 | 96.122 | 2.9440 | 51 | 0.3919 | 0.0405 | 0.2124 |
| 12 | 98.058 | 2.3114 | 27 | 97.839 | 2.1608 | 30 | -0.1220 | -0.1505 | 0.1334 |
| 13 | 99.556 | 0.4000 | 18 | 99.088 | 0.8327 | 27 | 0.4673 | 0.4327 | 0.0288 |
| 14 | 98.148 | 1.3311 | 15 | 97.569 | 1.3349 | 22 | 0.5783 | 0.0038 | 0.0114 |
| 15 | 97.712 | 1.8459 | 14 | 97.187 | 2.0938 | 23 | 0.5244 | 0.2479 | 0.1182 |
| 16 | 98.594 | 1.2078 | 16 | 96.682 | 2.5346 | 33 | 1.9124 | 1.3268 | 0.2266 |
| 17 | 97.479 | 2.0566 | 18 | 96.047 | 3.1060 | 25 | 1.4327 | 1.0495 | 0.1194 |
| 18 | 97.978 | 1.6178 | 15 | 97.705 | 1.6294 | 20 | 0.2729 | 0.0116 | 0.1606 |
| 19 | 98.196 | 1.1916 | 14 | 98.141 | 1.1386 | 18 | 0.0549 | -0.0530 | 0.1419 |
| 20 | 98.494 | 1.1129 | 15 | 98.220 | 1.1283 | 16 | 0.2739 | 0.0153 | 0.0766 |
| *Mean* | *98.50* | *1.0186* | *21* | *97.949* | *1.261* | *28* | *0.532* | *0.242* | *0.0939* |

*Multiple seed units that include at least one caryopsis with 1/3 the length of the palea are left intact and included in the pure seed fraction. Any floret with caryopses less than 1/3 the length of the palea is classified as inert matter.

Following the new rule, the average decrease in the amount of inert matter found to be 0.24 and 0.67% (Tables 1 and 2) compared to the AOSA rule (dismembering multiple florets

into pure seed and inert). Such dismembering artificially creates an inert matter that does not exist in the seed lot. The new pure seed definition will better comply with the AOSA definition of a seed unit as the "structure usually regarded as a seed in planting practices and in commercial channels", hence the Purity report will more accurately reflect the actual purity of the seed lot.

### *III. Comparison between the time required to complete purity tests using the proposed pure seed definition method and the current AOSA method for ryegrass and tall fescue.*

The results showed that the average time saved using the proposed pure seed definition over the current AOSA definition, is 18 and 7 minutes per sample for perennial ryegrass and tall fescue, respectively (Tables 1 and 2). The time saving was found to be only 2 minutes for annual ryegrass (data not shown). In general, as the amount of multiple seed units increases in a sample, the time saving using the proposed method over the current AOSA method will be greater

The study showed that adopting the proposed changes in the pure seed definition of ryegrass and tall fescue will increase testing objectivity, reduce variation in test results due to the number of processes involving breaking, separating, and weighing components of multiple seed units in the current AOSA rules, reduce the time required for purity testing of these species, and reduce discrepancies in purity testing results between AOSA and ISTA.

## REFERENCES


***Association of Official Seed Analysts. 2001. Rules for Testing Seeds. AOSA. Las Cruces, NM 88001.***

Elias, S. G. and A. Garay. (2002).Germination of perennial ryegrass seeds with caryopses less than one-third the length of the palea. (under revision foe publication).

***Everson, L. E. 1954. The germination of mature and immature seeds of quackgrass. Proc. Assoc. Off. Seed Anal. 44: 127-128.***

Penrose, J. 1997. Tall fescue germination on caryopses less than one-third the length of the palea. 1997-1998 Region 1 Northwest Referee, AOSA (Unpublished data).

# THE GERMINATION OF MATURE AND IMMATURE SEEDS OF QUACKGRASS (AGROPYRON RE PENS)!!

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Under the Association of Official Seed Analysts Rules for Testing Seed (1).~/ a quackgrass floret which by visual examination can be definitely demonstrated as having no embryo or only a rudimentary embryo is classed as inert matter. Mature quackgrass seeds are classed as weed seeds. How to determine whether an embryo is rudimentary or mature has been left to the discretion of the seed analyst. The term "rudimentary" is very indefinite; therefore, uniform classification of quackgrass "seeds" into weed seeds or inert matter by different seed analysts is unlikely. A standard which may be used to determine whether an embryo is rudimentary or mature is needed. Most seed analysts agree that such a standard should be based on potential ability of the embryo to germinate and develop into a seedling.

Dahlberg (2) investigated the germination of quackgrass seeds at different stages of maturity. He obtained a germination of 6% on seeds harvested 8 days after flowering and 48% on seeds harvested 12 days after flowering. However, he did not establish criteria which might be used for classification of these seeds. The study herein reported was initiated with the purpose of setting an arbitrary standard of classification based on the length of the caryopsis or grain in relation to the length of the palea.

## Material and Methods

During the summer of 1952, seed was hand harvested at Ames, Iowa at approximately weekly intervals from flowering to maturity of the seed. During the summer of 1953, quackgrass seed was similarly harvested at three locations--Lansing, Michigan; Fargo, North Dakota; and Ames, Iowa. The caryopsis and palea of each seed used for the germination test were measured under a dissecting microscope and the seeds classified Into four groups (as given in Table I. below) based on the degree of maturity. Fifty seeds were used for each germination test. The seeds were planted in petri dishes on a quartz sand substrate moistened with water. A temperature alternation of $15^0$C for 15 hours and $30^0$C for 9 hours was used. Germination was complete within 14 days.

## Results

These data indicate that seeds with caryopses shorter than 1/3 the length of the palea will not germinate and grow.

## Discussion and Conclusion

The only caryopses in classification 3 which germinated were those that were close to 1/2 the length of the palea. Caryopses shorter than 1/3 the length of the palea did not germinate, therefore, they should be considered immature or rudimentary. Inclusion of a specific statement to this effect in the Rules for Testing Seed should give analysts a reasonably consistent and realistic basis for classifying quackgrass seeds.

1/ Journal Paper No. 3-2522, of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 1083. This study was financed in part with funds appropriated under the Research and Marketing Act of 1946 and was carried out in cooperation with the Grain Division, Agricultural Marketing Service, United States Departni~nt of Agriculture.
2/ Refers to literature cited.

TABLE I. The percentage germination of quackgrass seeds at different stages of maturity.

| Source of Seed | 1953 Seed Lansing, Mich. | 1952 Seed Fargo, N. D. | Ames, Ta. | Ames, Ia | Average |
|---|---|---|---|---|---|
| 1. Caryopses over 1/2 the length of the palea - seeds plump. | 96 | 80 | 90 | 90 | 89 |
| 2. Caryopses over 1/2 the length of the palea but seeds shriveled. | 86 | 32 | 64 | 28 | 52 |
| 3. Caryopses from 1/3 to 1/2 the length of the palea. | 4 | 6 | 2 | 0 | 3 |
| 4. Caryopses less than 1/3 the length of the palea. | 0 | 0 | 0 | 0 | 0 |

Literature Cited

1. Association of Official Seed Analysts. Rules for Testing Seeds. Off. Seed Anal. 1949: 23-59. 1949.
2. Dahlberg, R. C. The germination of seeds of Agropyron repens. Off. Seed Anal. 1916: 21—24. 1916.

# TALL FESCUE GERMINATION ON CARYOPSES LESS THAN ONE-THIRD THE LENGTH OF THE PALEA

1997-98 Region 1 Northwest Referee

Jane Penrose, RST
Agri Seed Testing

## ABSTRACT

This project was sent to 32 seed labs with 24 of the them participating. Two samples of the same lot of tall fescue were sent with instructions on how to plant the seeds and a response sheet to fill out when evaluating the germination. One sample contained mature seeds while the other sample contained seeds with caryopses less than one-third the length of the palea. Of the 2500 seeds from the latter category, only one seed produced a seedling. These seeds are considered pure seed when following the AOSA Rules for Seed Testing, but are considered inert when following the rules for Canada and ISTA.

## INTRODUCTION

This referee is an extension of last years referee where the pure seed definition of tall fescue was examined. Canada and ISTA use the one-third rule to determine pure seed in the genera Lolium and Festuca. This year, germination tests were conducted on tall fescue seeds with caryopses less than one-third the length of the palea. Germination tests using fully mature seeds from the same lot of tall fescue were also conducted. The intent of this was to show lack of growth from the seeds in question was not a result of test conditions.

## PURPOSE

This referee was developed to examine the viability of tall fescue seeds with caryopses less than one-third the length of the palea.

## MATERIALS AND METHODS

Samples of tall fescue seed were sent to 32 seed labs. Two samples of the same lot were sent. One sample was fully mature seeds and the other sample contained seeds with caryopses with less than one-third the length of the palea. Participants were asked to plant 100 seeds of each sample following the germination method outlined which included: germination boxes, brown paper, KNO3, 15-25 degree Celsius germinator with light, 14 day final with no prechill. Two lots were used for this referee, one grown in 1996 and one grown in 1997. Each lab received one of these two lots for this study. Twenty-four seed labs completed and returned the project.

## RESULTS AND DISCUSSION

Samples 1-A and 1-B are from the same lot of tall fescue grown in 1996. Sample 1-A represents fully mature seeds, while sample 1-B are seeds with caryopses less than one-third the length of the palea. Tables 1 and 2 show the germination results for these two samples. The average germination for sample 1-A is 85 percent. Of the 1300 seeds planted from sample 1-B, only one seed germinated.
Samples 2-A and 2-B are from the same lot of tall fescue grown in 1997. Sample 2-A represents fully mature seeds, while sample 2-B are seeds with caryopses less than one-third the length of the palea. Tables 3 and 4 show the germination results for these two samples. The average germination for sample 2-A is 94 percent. Of the 1200 seeds planted from sample 2-B, no seeds germinated.

The purpose of samples 1-A and 2-A was as a control to demonstrate test conditions did not affect the germability of samples 1-B and 2-B. With the variability in test conditions that always occurs when conducting germination referee work, it was necessary to have mature seeds planted as well to show the potential of the seed lots.

## CONCLUSIONS

Seeds with caryopses less than one-third the length of the palea have little or no viability. When following the AOSA Rules for Seed Testing, these seeds are considered pure seed. They lower the germination percentage when present in a lot of seed. The germination percentage is an important measure of the quality of the seed lot. Canada and ISTA consider these to be inert. When following Canada and ISTA rules, the discount on the seed lot is in the pure seed percentage rather than in the germination percentage.

I would like to thank all the labs who participated.

Table 1

Sample 1 - A

| Lab # | Germination % | Abnormal % | Dead % | Notes: |
|---|---|---|---|---|
| 1 | 85 | 4 | 11 | |
| 2 | 89 | 0 | 11 | |
| 4 | 87 | 7 | 6 | blue blotters used |
| 5 | 76 | 10 | 14 | |
| 7 | 83 | 7 | 10 | 20-30° germinator |
| 8 | 88 | 3 | 9 | |
| 9 | 92 | 1 | 7 | 13 day final |
| 11 | 87 | 3 | 10 | blue blotters used |
| 12 | 86 | 3 | 11 | 13 day final |
| 13 | 81 | 0 | 19 | 18 day final |
| 14 | 84 | 2 | 14 | |
| 15 | 85 | 4 | 11 | |
| 32 | 83 | 5 | 12 | |

$\bar{X} = 85$

Table 2

Sample 1 - B

| Lab # | Germination % | Abnormal % | Dead % | Other % | Notes: |
|---|---|---|---|---|---|
| 1 | 0 | 0 | 100 | | |
| 2 | 0 | 0 | 100 | | |
| 4 | 0 | 0 | 0 | 100 - empty | blue blotters used |
| 5 | 0 | 0 | 100 | | |
| 7 | 0 | 0 | 100 | | 20-30° germinator |
| 8 | 1 | 0 | 99 | | |
| 9 | 0 | 0 | 96 | 4 - possible growth | 13 day final, moldy |
| 11 | 0 | 0 | 100 | | blue blotters used |
| 12 | 0 | 0 | 100 | | 13 day final |
| 13 | 0 | 0 | 100 | | 18 day final |
| 14 | 0 | 0 | 100 | | |
| 15 | 0 | 0 | 100 | | |
| 32 | 0 | 0 | 100 | | |

$\bar{X} = 0.00$ ....

Table 3

Sample 2 - A

| Lab # | Germination % | Abnormal % | Dead % | Notes: |
|---|---|---|---|---|
| 16 | 92 | 1 | 7 | |
| 18 | 92 | 2 | 6 | |
| 19 | 97 | 2 | 1 | layers of white filter paper used |
| 21 | 90 | 0 | 10 | 17-25°; 17 ° for 10 hours. 3 days in 25° non-consecutively |
| 22 | 98 | 0 | 2 | blue blotter used |
| 25 | 93 | 3 | 4 | |
| 27 | 94 | 0 | 6 | blue blotters used |
| 28 | 95 | 2 | 3 | 4 sheets brown towels used |
| 29 | 94 | 1 | 5 | 6 " petri dishes used |
| 30 | 94 | 2 | 4 | blue blotter used |
| 31 | 92 | 2 | 6 | 2 replicates of 50 seeds |
| 32 | 93 | 0 | 7 | |

$\bar{x} = 94$

Table 4

Sample 2 - B

| Lab # | Germination % | Abnormal % | Dead % | Other % | Notes: |
|---|---|---|---|---|---|
| 16 | 0 | 0 | 0 | | *no remarks made* |
| 18 | 0 | 0 | 100 | | slightly moldy |
| 19 | 0 | 0 | 0 | 100 - *no remarks* | layers of white filter paper used |
| 21 | 0 | 0 | 100 | | 17-25°; 17° for 10 hours. 3 days in 25° non-consecutively |
| 22 | 0 | 0 | 100 | | blue blotters used |
| 25 | 0 | 0 | 100 | | |
| 27 | 0 | 0 | 100 | | blue blotters; white-gray mold |
| 28 | 0 | 0 | 100 | | 4 sheets brown towels used |
| 29 | 0 | 0 | 100 | | 6" petri dishes used |
| 30 | 0 | 0 | 100 | | blue blotters used |
| 31 | 0 | 0 | 100 | | 2 replicates of 50 seeds |
| 32 | 0 | 0 | 100 | | |

$\bar{x} = 0$