Rule Change Proposal

PURPOSE OF PROPOSAL: To add a 'Uniform Blowing Procedure for tall fescue (*Festuca arundinacea*) to the AOSA Handbook Contribution No. 24 and the AOSA Rules for Testing Seeds; establish master calibration samples for tall fescue; and move tall fescue from PSU 21 to PSU 23.

PRESENT RULE:

Current wording in: AOSA RULES FOR TESTING SEEDS

2.7 Kind or cultivar considered pure seed. –

e. **Pure seed unit (PSU) definitions -** the seed units described in the following definitions shall be considered pure seed. Structures not specifically described as part of the PSU shall be removed and classified as inert matter (refer to section 2.10). The PSU numbers given for species in Table 1 correspond to the following PSU numbers. For species not listed in Table 1, use the PSU definition that best describes the species concerned.

PSU Number	Description of Pure Seed Unit
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.

2.11 Uniform blowing procedure

c. **Calibration samples:** The instructions stated in AOSA Handbook 24 shall be followed to determine the optimum calibration point for Kentucky bluegrass, orchardgrass and 'Pensacola' bahiagrass, and the equivalent air velocity value (m/s) using the air velocity calibration procedures. Blowers shall be calibrated prior to proceeding with the separation of pure seed and inert matter for the kinds requiring the uniform blowing procedure. The procedure is applicable only to General blowers. Standard calibration samples for all three species with instructions are available on a loan basis from the USDA Seed Regulatory and testing Branch, 801 Summit Crossing Place, Suite C, Gastonia, NC 28054-2193.

Current wording in: AOSA HANDBOOK 24 – The Uniform Blowing Procedure

4.1. General Blower.

The blower is operated in the normal manner, using a standard calibration sample (i.e., Kentucky bluegrass, orchardgrass or 'Pensacola' bahiagrass) on loan from the USDA Seed Regulatory and Testing Branch (see Sec. 2.3). For each blowing, set the timer for exactly three minutes. If you do not know approximately where you should begin your first blowing, it will be evident from each trial whether the gate opening is too high or low. For example, using Kentucky bluegrass, if there are many (20 or more) light florets left in the cup with the heavy fraction, then the air velocity is not high enough. Conversely, if numerous heavy florets are blown into the light fraction, then the setting is too high. Large changes in the dial setting should be used to quickly arrive at the approximate setting. Using the large tube, with bleeder valves closed, try settings of 6, 8, 10 and 12 for Kentucky bluegrass. When there are no more than 20 heavy florets in the light fraction, then you are near the correct setting. Continue the procedure, changing settings of the gate opening by 0.2 until the relative numbers of light and heavy seeds misplaced are about the same (0 to 10).

To gain accuracy, a series of blowings are made near the anticipated uniform blowing point. The number of misplaced seeds is averaged at each setting, and the data used to confirm the **optimum uniform blowing point**.

The data shown in Table 2 represents a series of blowings using a General blower, in which the calibration point is 10.80. It is evident that the average number of light florets in the heavy fraction is in excess at the 10.60 setting. The reverse is true at the 11.00 setting. At 10.80, the number of light and heavy florets are approximately equal.

Table 2. Trial blower settings using general blower to determine uniform blowing point.

Blower setting	Trial number	Light florets in heavy fraction	Heavy florets in light fraction
10.60	1	11	3
	2	13	5
	3	15	3
	Average	13	4
10.80	1	7	8
	2	7	5
	3	6	3
	Average	7	5
11.00	1	4	13
	2	5	15
	3	1	9
	Average	3	12

Blowers presently in use, and standard calibration samples prepared with grass florets have limited precision. The conscientious analyst who attempts to calibrate the blower precisely may become unnecessarily concerned by normal random variation in the number of misplaced florets. The data presented in Table 3 illustrates the normal random variation occurring at one setting.

Table 3. Variation in number of florets misplaced when repeatedly blown at one setting.

Trial number	Heavy florets misplaced	Light florets misplaced
1	3	2
2	6	7
3	6	5
4	8	9
5	5	6
6	9	5
7	5	2
8	7	4
9	6	2
10	5	8
Average	**6**	**5**

The number of heavy florets misplaced ranged from 3 to 9, the light florets from 2 to 9. The analyst that attempts trial blower settings for the General blower at increments smaller than 0.10 will observe the normal random variation that causes overlap in the number of florets misplaced at such precise settings. Once the optimum uniform blowing point for the calibration sample (i.e., Kentucky bluegrass, orchardgrass or 'Pensacola' bahiagrass) is established, determine the **equivalent air velocity value** (m/s) at that blower setting. To determine the equivalent air velocity value use a Turbo Wind Speed Meter, Model 271, Davis Instruments (or any similar model of rotary fan anemometer that will fit precisely over the opening at the base of the air stream column after the sample cup is removed), available for purchase at www.ambientweather.com/datume.html, or 877-413-8800 (or from any other source).

The following procedure shall be used to determine the equivalent air velocity value for a General Blower:

a. Turn on anemometer (check working condition of batteries), set digital air velocity display on meters per second (m/s).

b. Remove the sample cup from the cup holder of the blower, place the rotary fan anemometer with digital display facing up over the opening at the base of the air stream column. Align the fan precisely over the blower opening and hold in place.

c. Turn the blower on and read the air velocity value (m/s) when the blower has reached a steady running level (typically 30 seconds after turning on blower) and continue reading for 30-60 seconds. The value that appears most frequently on the digital display is recorded as the equivalent air velocity value for the calibrated optimum blowing point. For example, the digital display at the steady running level of the blower may show 2.5 m/s most frequently and fluctuate between 2.4 to 2.6 occasionally. In this case, the equivalent air velocity value is recorded as 2.5 m/s ± 0.1.

d. This equivalent air velocity value is valid only for this particular optimum blowing point (for a particular species, e.g., Kentucky bluegrass) for this particular General blower. Different General blowers within a laboratory must be calibrated independently.

Subsequent General blower calibrations for blowers with an established equivalent air velocity value can be rechecked following steps a through d. Adjust the blower gate opening until the air speed indicated on the anemometer digital display is equal to the equivalent air velocity value for the kind of seed being tested.

For species requiring factored adjustments of the optimum uniform blowing point for Kentucky bluegrass (i.e., rough bluegrass, blue grama, side-oats grama and weeping alkaligrass), multiply the optimum uniform blowing point (i.e., blower gate opening setting) by the appropriate factor. The product is the optimum uniform blowing point for the kind under consideration. Next set the blower gate opening at the optimum uniform blowing point for the kind under consideration and determine the equivalent air velocity value (m/s) for that setting following steps a through d. Subsequent General blower calibrations for blowers with an established equivalent air velocity value can be rechecked following steps a through d. Adjust the blower gate opening until the air speed indicated on the anemometer digital display is equal to the equivalent air velocity value for the kind of seed being tested.

PROPOSED RULE:

Proposed wording for: AOSA RULES FOR TESTING SEEDS

2.7 Kind or cultivar considered pure seed. –

e. **Pure seed unit (PSU) definitions -** the seed units described in the following definitions shall be considered pure seed. Structures not specifically described as part of the PSU shall be removed and classified as inert matter (refer to section 2.10). The PSU numbers given for species in Table 1 correspond to the following PSU numbers. For species not listed in Table 1, use the PSU definition that best describes the species concerned.

PSU Number	Description of Pure Seed Unit
23	Multiple floret spikelet, multiple floret, or floret, with or without pedicel, with or without awn(s), caryopsis, or piece of broken caryopsis larger than one-half of the original size remaining in the heavy portion following the Uniform Blowing Point Procedure in section 2.11. Special consideration: * For *Bouteloua curtipendula*, in addition to the units described above, spikelet group that disarticulates as a unit with attached rachis and internode.

2.11 Uniform blowing procedure

c. **Calibration samples:** The instructions stated in AOSA Handbook 24 shall be followed to determine the optimum calibration point for Kentucky bluegrass, orchardgrass, tall fescue and ‘Pensacola’ bahiagrass, and_the equivalent air velocity value (m/s) using the air velocity calibration procedures. Blowers shall be calibrated prior to proceeding with the separation of pure seed and inert matter for the kinds requiring the uniform blowing procedure. The procedure is applicable only to General blowers. Master calibration samples for all four species with instructions are available on a loan basis from the USDA Seed Regulatory and testing Branch, 801 Summit Crossing Place, Suite C, Gastonia, NC 28054-2193.

d.

(9) **Tall fescue:** The equivalent air velocity value (m/s) for the optimum calibration point obtained with the tall fescue master calibration sample shall be used.

Proposed wording for: AOSA HANDBOOK 24 – Uniform Blowing Procedure

2. THE CALIBRATION SAMPLE

2.3. Source.

Kentucky bluegrass, orchardgrass, tall fescue and Pensacola bahiagrass calibration samples are available only through loan. The loaned samples must be returned promptly to the USDA Seed Regulatory and testing Branch, 801 Summit Crossing Place, Suite C, Gastonia, NC 28054-2193 after a laboratory has completed their blower calibration. Periodically, the Association should check the master calibration samples for deterioration.

2.4. Preconditioning.

If the master calibration sample is kept in a desiccator or under a temperature-humidity condition different from the ambient laboratory conditions, the calibration sample must be pre-conditioned. The purpose of pre-conditioning is to bring the moisture content of the calibration sample into equilibrium with atmospheric moisture. The calibration sample should be exposed to open room atmosphere for approximately sixteen hours or overnight before using. Care should be taken so that the sample is not spilled or otherwise damaged when left in an open container.

4. BLOWER CALIBRATION

4.1. General Blower.

The blower is operated in the normal manner, using a master standard calibration sample (i.e., Kentucky bluegrass, orchardgrass, tall fescue or ‘Pensacola’ bahiagrass) on loan from the USDA Seed Regulatory and Testing Branch (see Sec. 2.3). For each blowing, set the timer for exactly three minutes. If you do not know approximately where you should begin your first blowing, it will be evident from each trial whether the gate opening is too high or low. For example, using Kentucky bluegrass, if there are many (20 or more) light florets left in the cup with the heavy fraction, then the air velocity is not high enough. Conversely, if numerous heavy florets are blown into the light fraction, then the setting is too high. Large changes in the dial setting should be used to quickly arrive at the approximate setting. Using the large tube, with bleeder valves closed, try settings of 6, 8, 10 and 12 for Kentucky bluegrass. When there are no more than 20 heavy florets in the light fraction, then you are near the correct setting. Continue the procedure, changing settings of the gate opening by 0.2 until the relative numbers of light and heavy seeds misplaced are about the same (0 to 10).

To gain accuracy, a series of blowings are made near the anticipated uniform blowing point. The number of misplaced seeds is averaged at each setting, and the data used to confirm the **optimum uniform blowing point**.

The data shown in Table 2 represents a series of blowings using a General blower, in which the calibration point is 10.80. It is evident that the average number of light florets in the heavy fraction is in excess at the 10.60 setting. The reverse is true at the 11.00 setting. At 10.80, the number of light and heavy florets are approximately equal.

Table 2. Trial blower settings using general blower to determine uniform blowing point.

Blower setting	Trial number	Light florets in heavy fraction	Heavy florets in light fraction
10.60	1	11	3
	2	13	5
	3	15	3
	Average	13	4
10.80	1	7	8
	2	7	5
	3	6	3
	Average	7	5
11.00	1	4	13
	2	5	15
	3	1	9
	Average	3	12

Blowers presently in use, and standard calibration samples prepared with grass florets have limited precision. The conscientious analyst who attempts to calibrate the blower precisely may become unnecessarily concerned by normal random variation in the number of misplaced florets. The data presented in Table 3 illustrates the normal random variation occurring at one setting.

Table 3. Variation in number of florets misplaced when repeatedly blown at one setting.

Trial number	Heavy florets misplaced	Light florets misplaced
1	3	2
2	6	7
3	6	5
4	8	9
5	5	6
6	9	5
7	5	2
8	7	4
9	6	2
10	5	8
Average	**6**	**5**

The number of heavy florets misplaced ranged from 3 to 9, the light florets from 2 to 9. The analyst that attempts trial blower settings for the General blower at increments smaller than 0.10 will observe the normal random variation that causes overlap in the number of florets misplaced at such precise settings. Once the optimum uniform blowing point for the calibration sample (i.e., Kentucky bluegrass, orchardgrass, tall fescue or 'Pensacola' bahiagrass) is established, determine the **equivalent air velocity value** (m/s) at that blower setting. To determine the equivalent air velocity value use a Turbo Wind Speed Meter, Model 271, Davis Instruments (or any similar model of rotary fan anemometer that will fit precisely over the opening at the base of the air stream column after the sample cup is removed), available for purchase at www.ambientweather.com/datume.html, or 877-413-8800 (or from any other source)....(the remaining paragraphs of this section are unchanged)

5. UINIFORM BLOWING METHOD

5.7. Tall fescue *(Festuca arundinacea).*

The equivalent air velocity value (m/s) for the optimum calibration point obtained with the tall fescue master calibration sample shall be used.

HARMONIZATION STATEMENT

The uniform blowing procedure has been shown to provide accurate and repeatable results for Kentucky bluegrass, orchardgrass, weeping alkaligrass, and other grass species and is easy to apply and harmonize in seed laboratories. The proposed blower calibration method using master calibration samples and air velocity calibration can easily be adapted to other seed testing rules, such as Federal Seed Act, ISTA and CFIA Methods and Procedures. The proposed seed unit for tall fescue will include multiple seed units and therefore will be similar to the seed unit described in the ISTA Rules. Although ISTA and CFIA require the caryopsis to be 1/3 the length of the palea for this species (based on subjective visual examination), the length of caryopses will not be a harmonization issue because the blowing procedure removes small non-viable or weak germinating caryopses.

SUPPORTING EVIDENCE

Summary of research:

The current method for separation of light inert in tall fescue depends on the visual interpretation of the length of the caryopsis within the floret (one-third the length of the palea as described under PSU 21) and does not have the necessary controls to assure uniformity among analysts and laboratories. The current AOSA method also requires identifying multiple florets, dismembering them, determining which florets contain or do not contain seeds, and separating them into pure seed and inert matter. In addition to the time and cost of labor, this procedure is subjective and lends itself to inconsistencies in test results among laboratories. In the end, this is a problem for both seed testing labs and grass seed industry.

The multiple seed units are currently regarded and handled as seed units in typical planting practices and commercial channels by growers, seed dealers, and seed trade. Thus, assessing the planting value of multiples in a manner that reflects the true condition as they are in the bag would be more realistic.

The uniform blowing procedure has been universally accepted as the most efficient and repeatable method for purity analysis of Kentucky bluegrass, Canada bluegrass, rough bluegrass, weeping alkaligrass, orchardgrass, blue grama and side oats grama. In June 2006, the Association of Official Seed Analysts and the Society of Commercial Seed Technologist adopted a new method for calibrating General-type blowers used for the uniform blowing procedure for orchardgrass and Kentucky bluegrass. A national referee study showed that the new method achieved better uniformity in calibrating different blowers within and among laboratories and increased uniformity in test results compared to the old calibration method for orchardgrass and Kentucky bluegrass. The new method proved to be effective and simple to use and was used as a model for developing an efficient and objective blowing procedure of tall fescue.

To demonstrate the feasibility and uniformity of the proposed blowing procedure for tall fescue, the research was conducted in several stepwise stages to answer two questions: 1) can the uniform blowing procedure adequately separate inert matter (i.e., florets without caryopses and of no planting value) from pure seed (i.e., florets containing caryopses of planting value), and 2) whether single florets and multiple florets behave differently under the same blowing conditions. The first two studies were carried out on both single and multiple florets as defined in sec. 2.12. The next three studies (3-5); were conducted on multiple seed units (MSU) only as defined in sec. 2.12; and the last study is an internal referee on both single and multiple seed units.

Following is a summary of the studies:

1. Understanding the principle. It has been demonstrated that there is a point (measured by air-gate opening and its equivalent air velocity) at which, light-weight inert matter can be blown out. This principle was demonstrated by visual observations based on the caryopsis length (1/3 rule as described in PSU 21) as well as by germination.

2. Validating across samples. The identified optimum separation point for TF was used to test 100 random samples. This stage demonstrated that the chosen point lifted mainly light inert matter to the blowing pan and left the pure seeds in the seed cup of the blower. A germination study further confirmed that the light portion from the blowing procedure either did not germinate or germination was negligible and the seedlings looked small and weak. This stage also made it possible to settle for an optimum blowing point to be used as the reference for the next stage.

3. Determining the frequency of multiple florets in tall fescue samples.

4. Measuring the effectiveness of the blowing procedure in separating lightweight multiples (with no planting value) from multiples in the heavy fraction (with planting value).

5. Validating the effectiveness of the blowing procedure in separating lightweight multiples from heavy multiples (with planting value) from samples containing very low to very high number of multiples.

6. Application for testing. Calibration samples of proven uniformity were developed using the optimum calibration point for tall fescue that was developed in the previous two stages. Such a calibration sample was used to test the repeatability in test results across seven General blowers (internal referee) using blind samples. This study demonstrated that all blowers produced similar results in separating light inert matter from pure seed regardless of the initial percentage of inert matter in samples. This stage will be followed up by a national referee study (in preparation) to test uniformity across laboratories.

In essence, these stepwise studies demonstrated that a uniform blowing procedure can achieve consistent results in separation of light-weight inert matter from pure seed in TF. The protocol would be the same as in orchardgrass and Kentucky bluegrass listed in Handbook No. 24. The General blowers would be calibrated using tall fescue master calibration samples of proven uniformity to determine the optimum blowing point. The equivalent air velocity (EAV) would be measured using an anemometer (Model 271, Davis Instruments, CA). This EAV value would be used to calibrate (reproduce the blowing point) subsequently to test samples. The master calibration sample would be needed again only if the blower changes physically (e.g., changing the motor, the fan, or the glass tube).

Details of the sequential studies that have been conducted to develop the standard blowing procedure for tall fescue are presented below:

1. Demonstration of the separation principle.

a. Determining the blowing point for tall fescue by visual assessment

The following procedure was used to determine the optimum blowing point to separate light inert matter from pure seeds. Three tall fescue samples with different levels of inert matter (approximately 1, 2, and 4%) were used and repeated across seven General blowers at the Oregon State University Seed Laboratory.

1. Five grams from each sample was blown for three minutes at increasing levels of air velocities (starting from low air velocity of 2.3m/s and increasing gradually up to 3.2m/s). Each fraction was assessed for number of pure seed and inert matter content using the current rule (caryopses with less than 1/3 the length of the palea were considered inert matter; and 1/3 or more pure seed). This study was repeated in seven blowers to determine if there was a common separation point regardless of the sample condition in any blower.

2. The results were used to develop a standard curve for each sample in each of the seven blowers to identify an optimum point. The criterion for determining the optimum blowing point was to identify the crossover point, i.e., a point where the maximum numbers of inert florets were blown out and a minimum number of pure seeds were lost.

Figure 1 presents an example (blower number 4) of what has been observed. This example demonstrates that there is a point at which light inert matter can be separated from pure seeds making sure that a minimum number of pure seeds are lost along with the blowing portion and a minimum or none of empty seeds are left in the heavy pure seed portion. The results showed that regardless of the amount of light-weight inert matter in a sample, the optimum separation point occurred at the same air velocity value, a condition which is critical to develop a blowing point that is applicable for all samples. The same phenomenon was observed in the seven blowers.

b. Confirming the optimum blowing point for tall fescue by germination.

In this study, each light portion separated by blowing at 2.3, 2.6, 2.9, 3.2, 3.5, 3.8, 4.1, and 4.3 m/s, in addition to check control (seeds remaining in the pure seed cup), were germinated using the AOSA rules to determine if the light portion contained germinable seeds.

This process was performed for the same three samples used in the first study and repeated in all the seven blowers. Figure 2 presents an example obtained in one blower (No. 4). The results demonstrated that seeds from the light portion that were blown out below 3.2 m/s had very low germination, while the remaining heavy pure seed portion germinated around 90% or above. These two studies demonstrated that a point between 2.9 and 3.1 would be a suitable blowing point because it would blow out mainly or only inert matter without any planting value as demonstrated by visual assessment and by germination.

Fig. 1. Empty and pure seeds blown out at various air velocity settings from three tall fescue samples (containing 1, 2 and 4% inert material from left to right) using a general blower. The arrows show the crossover points, which are suitable blowing points.

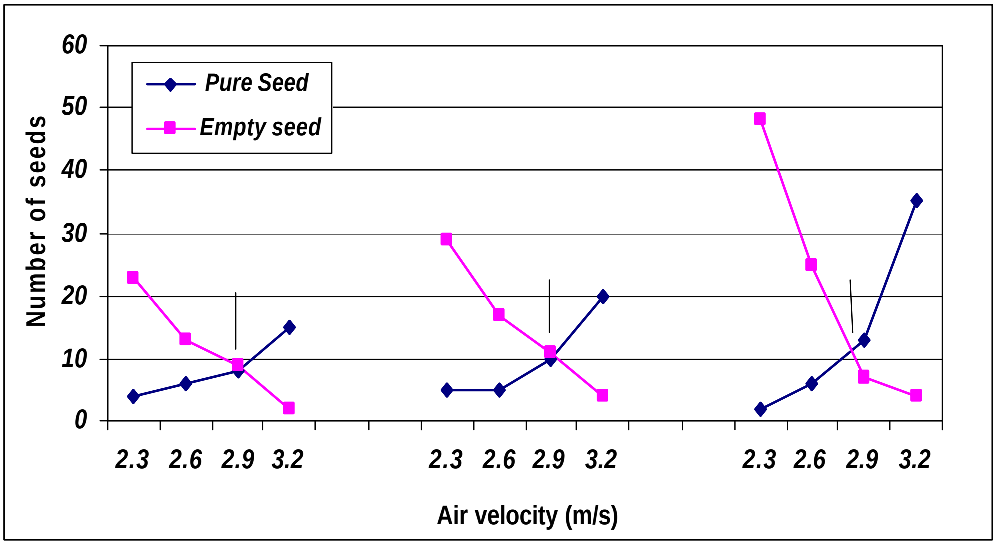
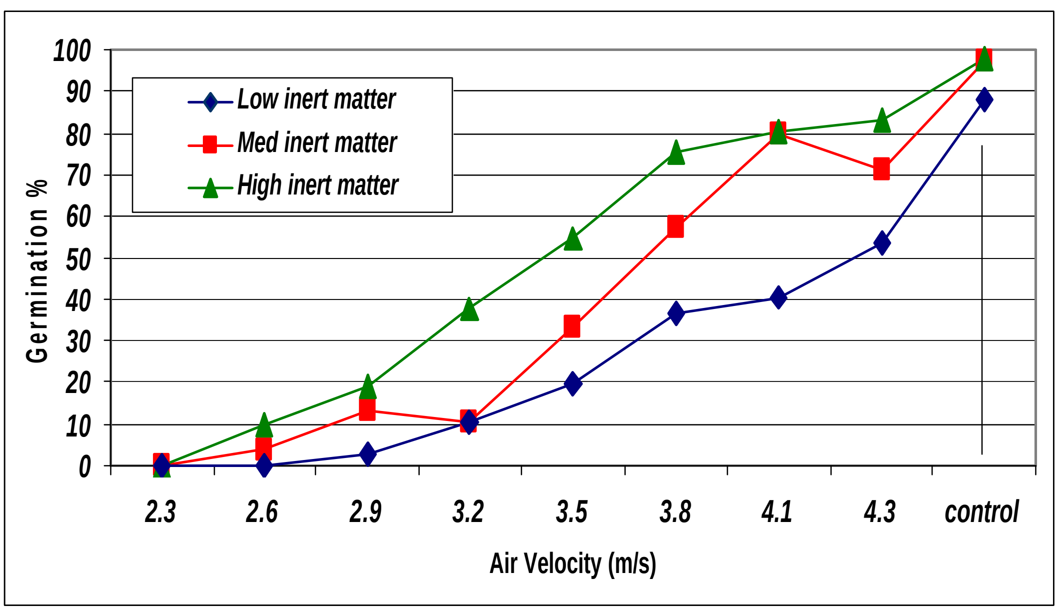


Fig. 2. Germination percentage of TF light portions blown at different air velocity values in a General blower as compared to the control (the heavy pure seed portion).



2. Validation studies across 100 random tall fescue samples

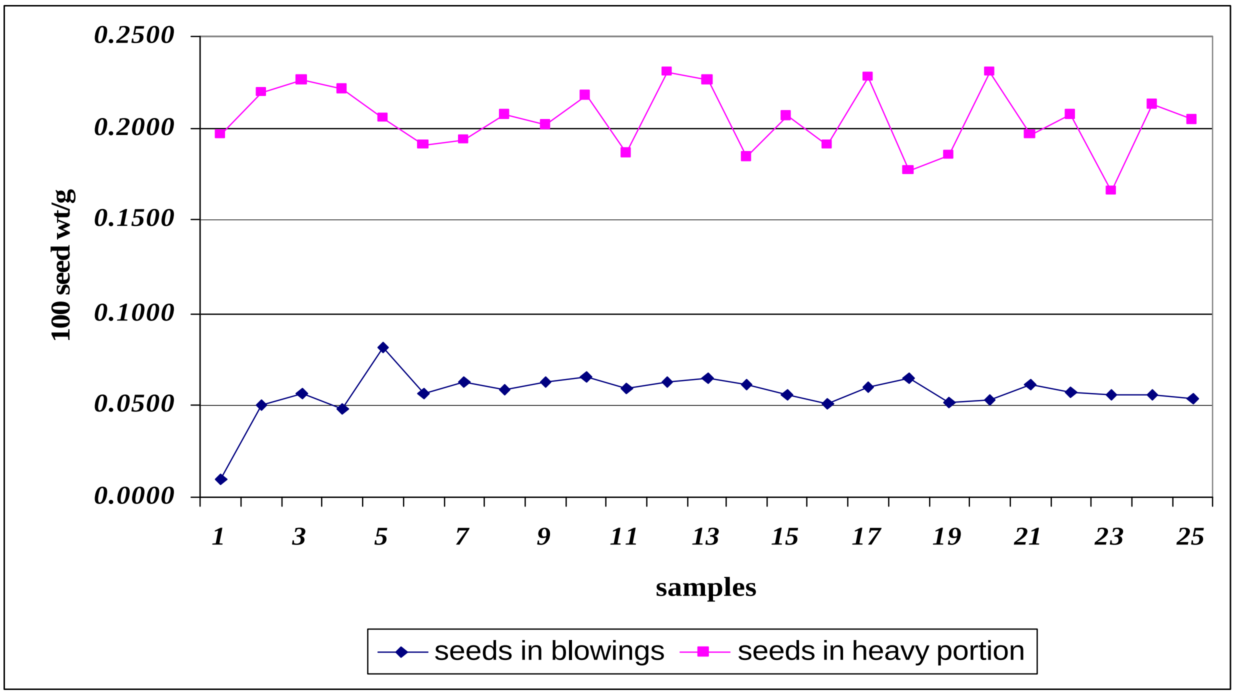
The objective of the studies in phase 2 was to validate the effectiveness of the blowing point (e.g., 3.0 m/s in blower No. 4) determined in the previous studies in separating light-weight empty florets from pure seeds in large number of samples (e.g., 100).

a. Validation by visual assessment.

One hundred random tall fescue samples representing different varieties and sample conditions were used for this study. Each sample was thoroughly homogenized and 5g were taken from each sample and placed in envelopes marked with the sample number. Each sample was blown for 3 min at the identified blowing point. The heavy portion and the light portion were placed in separate envelopes and marked with the same number. Visual separation of each portion was performed to determine the number of pure seeds present (by the 1/3 rule) in the blowing portion. Weight of 100 seeds from the light and the heavy portion of 25 random tall fescue samples were determined.

The results showed that the majority of the florets blown out were completely empty and a very small proportion, 0.4% of the blowings, contained borderline seeds showing one-third or more endosperm development. The results also showed that the weight of the seed like structures in the blowings is significantly less than seed in the heavy portion, approximately, 4 light**:** heavy seed ratio (Fig. 3)

Fig. 3. Weight of 100 seeds from the light and the heavy portion of 25 tall fescue samples using the proposed standard blowing procedure.

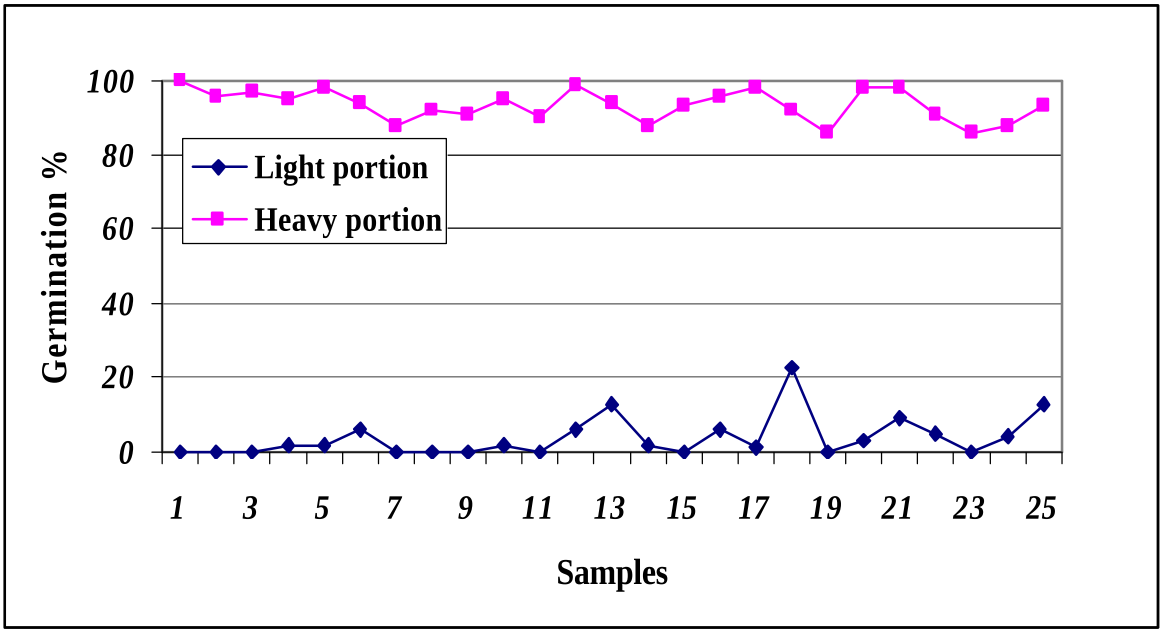


b. Validation by germination.

This study was conducted on 25 out of the 100 samples used in the previous study representing sample conditions ranging from very small amounts in the light portion (initial light inert matter content) to the highest amount of material in the light portion. This stratified study was necessary to determine if germinable seeds were being discarded as inert matter, especially in those samples that showed high amounts of material in the light portion. One-thousand seed weight of submitted samples was determined as well.

The average germination of the 25 samples from the light portion was 4.1% compared to 92.2% in the heavy portion that remained in the pure seed cup (Fig. 4 and Table 1). Figure 5 presents an example for five samples that represent an average sample in terms of number of blowings. These results confirmed what has been observed visually and demonstrated that the chosen point (EAV value 3.0 m/s in blower 4) was effective in separating light-weight inert matter from heavy pure seeds. Furthermore, it demonstrated its effectiveness in samples containing small amounts of material in the light portion as well as in samples that have high amounts. The 1000-seed weight of the 25 samples ranged from 1.75g to 2.28g. This showed that samples represented a wide range of environments and physiological qualities.

Fig. 4. Germination of light and heavy portions of 25 tall fescue samples following the blowing procedure.



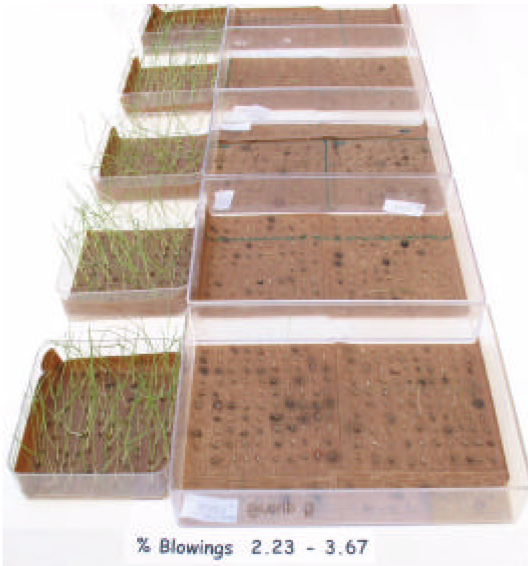


Fig. 5. Germination of the light portion and the heavy portion seeds of five tall fescue samples blown at 3.0 m/s air velocity. The average germination percentage of the light portion of 25 samples was 4.1% compared to 92.2% in the heavy portion.

Table 1. One-thousand seed weight, germination of seed like structures in the light portion and in the heavy portion of 25 tall fescue samples blown using the proposed blowing procedure.

Sample No.	***1000 seed weight (singles and multiples)***	**Blowing**		**% Germination of 100 singles & multiples in Heavy portion**
		Total No. singles + multiples in blowings	**Number germinated**	
1	2.20	0	0	100
2	2.16	1	0	96
3	2.24	3	0	97
4	2.24	10	2	94
5	2.15	12	2	98
6	2.01	26	6	94
7	2.07	25	0	87
8	2.09	35	0	90
9	2.05	35	0	91
10	2.17	43	2	95
11	1.87	57	0	88
12	2.26	60	6	100
13	1.91	63	13	87
14	2.10	68	2	87
15	2.01	75	0	89
16	1.99	94	6	95
17	2.28	89	1	98
18	1.75	108	23	92
19	2.01	166	0	79
20	2.27	169	3	98
21	1.84	184	9	96
22	1.76	186	5	88
23	1.84	204	0	86
24	2.02	324	4	88
25	1.91	329	13	91
Mean	**2.05**			**92.2**
Total		**2366**	**97**	

3. Frequency of multiple florets present in tall fescue samples.

Critical information to understand the behavior of multiple seed units (MSU) in tall fescue should start by measuring their frequency in samples. Repeated observations in past years indicate that tall fescue samples typically do not contain large number of MSU. The objective of this study was to measure the magnitude of the MSU present in tall fescue. To have robust information, all samples that arrived randomly in the middle of the tall fescue testing season (August of 2006), totaling 336 samples were evaluated. The samples represented many varieties, field conditions, cleaning conditions, levels of MSU content in each sample. Each sample was homogenized and divided using the AOSA procedure to obtain the 5g test sample from each submitted. Seeds were placed in envelopes marked with the sample number. The number of MSU's in each individual sample was determined according to sec. 12.2 in the AOSA Rules.

To understand clearly the frequency of MSU found in each sample, the results were ranked from the lowest to the highest as shown in Figure 6. These results showed the majority of tall fescue samples contain low numbers of MSU; of the 336 samples tested, 320 (95%) had less than 50 multiples (Fig. 7); i.e., less than 2% (by number) in a sample are multiples. This confirmed observations made in the past by many analysts in the OSU laboratory. The implication of this finding is that the risk of introducing a meaningful error by using the new method will be very low because the low numbers of MSU in the sample does not leave much room for significant errors. Furthermore, most MSU that do not contain seeds would be blown out and considered inert matter.

Fig. 6. Number of multiple florets found in 336 random tall fescue samples ranked from lowest to highest.

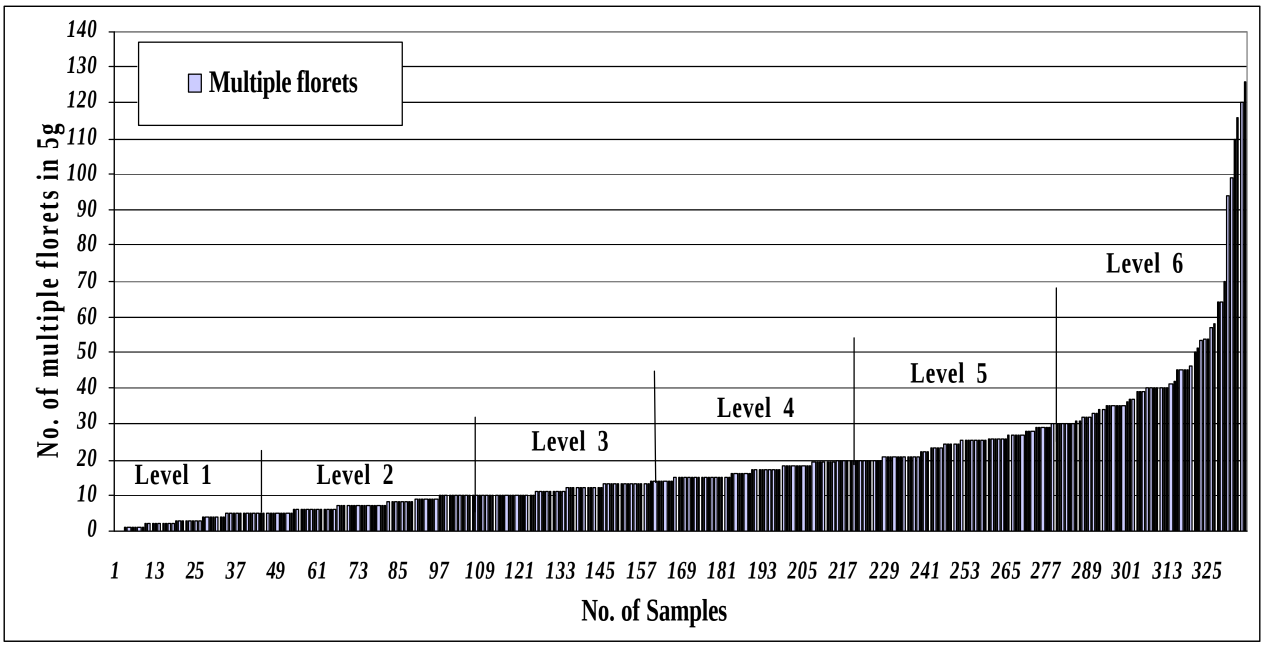
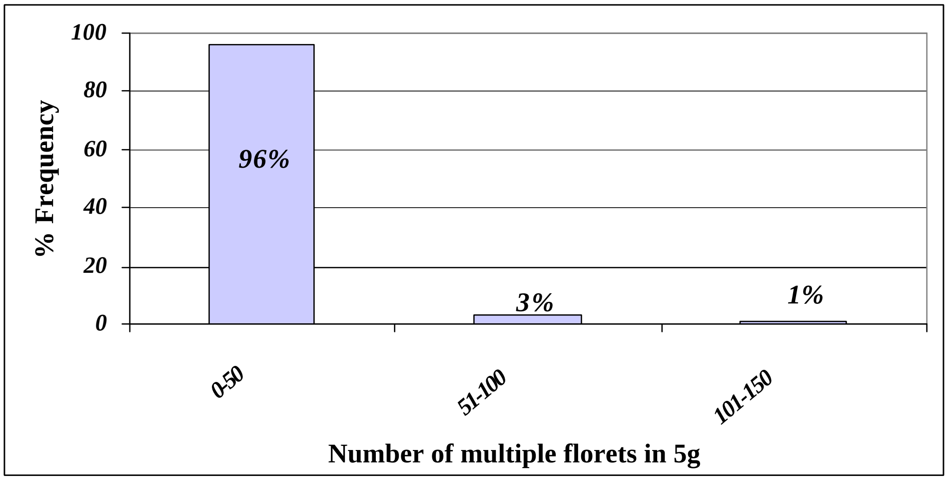


Fig. 7. Relative frequency of multiple florets found in 336 random tall fescue samples.



4. Measure the effectiveness of the blowing procedure in separating light and heavy MSU.

The study objective was to determine whether the blowing point used in this proposal could adequately separate lightweight MSU (without planting value) from MSU (with planting value) in tall fescue samples. This approach was studied based on the theory that multiple florets that do not contain caryopses have less density than those that contain developed caryopses. In theory, each type of MSU behaves differently in the presence of air current in a blower.

For a robust test, samples that contained high numbers of MSU were identified from each of 2002, 2003 and 2004 crop years. Ten samples from each year representing different growing seasons, varieties and cleaning conditions were used. The samples were homogenized, divided to obtain the 5 grams as required by the AOSA Rules and blown for 3 min at the optimum blowing point. The number of MSU in the light fraction (blowings) and in the heavy fraction was counted separately.

The results showed that MSU in the light portion rarely contained caryopses by visual observation based on the (1/3 rule), in contrast most MSU in the heavy portion contained caryopses.

For further assessment of the planting value, the MSU from the light and heavy portions were planted separately according to the AOSA Rules for Testing Seeds. The final germination of MSU in each fraction was evaluated (Table 2). Results indicate MSU from the light portion had no or minimal germination, whereas most MSU in the heavy fraction did contain caryopses with high planting value. Only 12 out of a total of 589 (2%) MSU from the light portion germinated; in contrast, 988 out of 1669 (59.2%) MSU in the heavy portion germinated. This evidence suggests the blowing procedure can be an effective tool to assess the planting value of MSU without a need to break them apart and separate them into inert and pure seed. Furthermore, this procedure would reflect the actual MSU present in seed bags and used in the planting practices.

Table 2. Germination of multiple seed units (MSU) found in the light portion and in the heavy portion as separated by the blowing procedure in 10 tall fescue samples from three production years (2002, 2003, and 2004).

Sample Year	*TF Sample*	*Total No. MSU in sample*[1]	Light Portion (Blowings)			Heavy Portion (HP)		
			No. of MSU in blowings	No. germinated	Average % germ . of all MSU's in blowings	No. of MSU in HP	No. germinated	Average % germ of MSU's in HP
2002	1	130	10	0		120	82	
	2	31	16	0		15	8	
	3	106	20	0		86	43	
	4	85	32	1		53	28	
	5	84	29	0		55	18	
	6	61	10	0		51	23	
	7	71	35	0		36	20	
	8	99	27	1		72	47	
	9	160	24	1		136	52	
	10	84	31	0		53	20	
2003	1	23	7	0		16	11	
	2	174	68	0		106	47	
	3	83	20	1		63	35	
	4	101	24	0		77	47	
	5	100	3	1		97	83	
	6	125	38	0		87	39	
	7	69	26	0		43	20	
	8	135	15	0		120	64	
	9	68	29	0		39	27	
	10	44	10	0		34	13	
2004	1	21	3	0		18	15	
	2	50	1	0		49	45	
	3	51	23	1		28	22	
	4	18	6	0		12	12	
	5	24	10	0		14	14	
	6	40	15	0		25	20	
	7	34	8	1		26	24	
	8	23	6	2		17	14	
	9	119	26	3		93	74	
	10	45	17	0		28	21	
	Total		**589**	**12**	**2.0**	**1669**	**988**	**59.2**

[1] Number of multiple florets in 5 gram sample.
[2] MSU = Multiple Seed Units (multiple florets).
[3] HP = Heavy portion.

5. Validate the effectiveness of the blowing procedure in separating lightweight MSU (without planting value) from heavy MSU (with planting value) from samples containing very low to very high numbers of MSU in 2006.

This study was designed to validate the effectiveness of the proposed blowing procedure in separating lightweight MSU from thirty samples containing various numbers of multiples (Table 3).

Five random samples from each MSU frequency level (see the six different levels in Fig.7) was obtained for a total of 30 samples out of the 336 samples used in Study 4. The stratified study made it possible to include extreme samples with few MSU as well as those with large numbers of MSU. This was necessary to determine whether the proposed blowing procedure would be effective regardless of the number of MSU present in the sample.

All samples used in this study were from the 2006 growing season. They represented various varieties, growing environments and cleaning conditions. Five grams from each sample was blown for 3 min at the optimum blowing point following the procedure described in Handbook No. 24. The MSU in the light portion and in the heavy portion were weighed and were germinated following the AOSA Rules for Testing Seeds. In addition, 100 seeds from the heavy portion of each of the 30 samples were germinated as control checks. The final germination percentages of each fraction was determined.

Results showed that out of the 306 MSU from the light portions of all 30 samples, only 43 contained caryopses (14.1%) based on visual observation and of those only 5 germinated (1.6%), demonstrating these kinds of MSU can be considered inert matter. In contrast, out of 1251 MSU found in the heavy portion, 1073 contained caryopses (85.8%) based on visual observation and of those 772 germinated representing 61.7%. This evidence indicates that MSU remaining in the heavy portion and have planting value. It is questionable whether breaking them up into single florets and inert matter would not contribute to any significant increase in the value of the information.

It is evident that the blowing procedure is just as effective in separating empty multiples from multiples containing seeds regardless of the number of total multiples present in the sample. The study also demonstrated that the method is as effective in samples with low or with high numbers of multiples.

Table 3. Weight and germination of multiple seed units (MSU) found in the light portion and in the heavy portion of 30 tall fescue samples (2006) separated by the blowing procedure.

TF Sample[1]	*Total No. MSU in samples*	Light Portion (Blowings)					Heavy Portion					% Germ of control (HP seed)
		% by wt of MSU in blowings	No. of MSU[2] in blowings	No. MSU containing pure seed	No. germinated	% germ on MSU in all blowing	% by wt of MSU in HP[3]	No. of MSU in HP	No. MSU containing pure seed	No. germinated	% germ on MSU in HP	
1	1	0.00	0	0	0		0.03	1	1	1		94
2	3	0.00	0	0	0		0.10	3	3	3		97
3	6	0.05	4	1	0		0.06	2	2	1		95
4	7	0.00	0	0	0		0.26	7	7	6		96
5	7	0.05	2	1	0		0.14	5	3	2		95
6	7	0.00	0	0	0		0.20	7	7	3		92
7	12	0.09	5	0	0		0.22	7	5	4		94
8	15	0.06	3	0	0		0.45	12	9	9		95
9	16	0.03	2	0	0		0.49	14	12	11		94
10	20	0.08	4	0	0		0.74	16	14	11		99
11	21	0.15	11	3	1		0.30	10	10	9		92
12	23	0.01	1	0	0		0.75	22	21	12		89
13	31	0.11	7	1	0		1.09	24	22	17		93
14	35	0.00	0	0	0		1.50	35	34	27		91
15	35	0.02	1	0	0		1.51	34	30	18		93
16	39	0.20	13	2	0		1.04	26	23	16		93
17	40	0.07	4	0	0		1.22	36	34	25		91
18	42	0.09	5	2	0		1.49	37	36	26		92
19	48	0.13	8	1	0		1.53	40	39	34		94
20	55	0.04	3	1	0		2.13	52	44	35		96
21	66	0.17	10	3	1		1.97	56	51	36		88
22	68	0.38	20	1	0		2.57	48	42	28		91
23	85	0.11	6	2	2		2.89	79	64	43		85
24	86	0.31	20	2	0		2.44	66	51	45		94
25	91	0.25	18	4	0		2.63	73	65	54		89
26	118	0.44	23	5	0		3.73	95	90	61		86
27	120	0.23	14	3	0		3.95	106	90	59		81
28	124	0.44	31	1	0		3.00	93	79	39		78
29	126	0.47	27	4	0		3.32	99	75	44		91
30	210	1.01	64	6	1		5.16	146	110	93		94
Total			306	43	5	**1.6**		1251	1073	772	**61.7**	**91.7**

[1] Sample size 5.1 - 5.3g
[2] MSU = Multiple Seed Units (multiple florets).
[3] HP = Heavy portion.

6. Applicability of tall fescue blowing procedure for seed testing

The application of the new blowing procedure for tall fescue in seed testing laboratories depends on the availability of calibration samples of proven uniformity, and on producing consistent results across blowers. Thus, phase 3 focuses on the following studies:

a) Development of master calibration samples for tall fescue

Since no standard calibration samples of tall fescue exist, a study was conducted to understand the principles of standard calibration samples, analyze the information produced from the studies in phase 1 and 2, and to prepared detailed procedure to produce uniform calibration samples for tall fescue. The calibration samples are designed to locate the optimum blowing point in various General blowers. Nine calibration samples of proven uniformity (master calibration samples) were produced (Fig. 8) to make sure there would be enough calibration samples for the national referee study and to make it available for the AOSA if and when the rule proposal is accepted.

The specific procedure for producing the uniform calibration samples will be published separately in 'Seed Technology' in the near future to stimulate interest in preparing blowing procedures for other species.

Fig. 8. Nine standard calibration samples with proven uniformity were prepared at Oregon State University Seed Laboratory to be used for calibrating General blowers to determine the optimum blowing point for tall fescue.

b) Testing for uniformity across blowers.

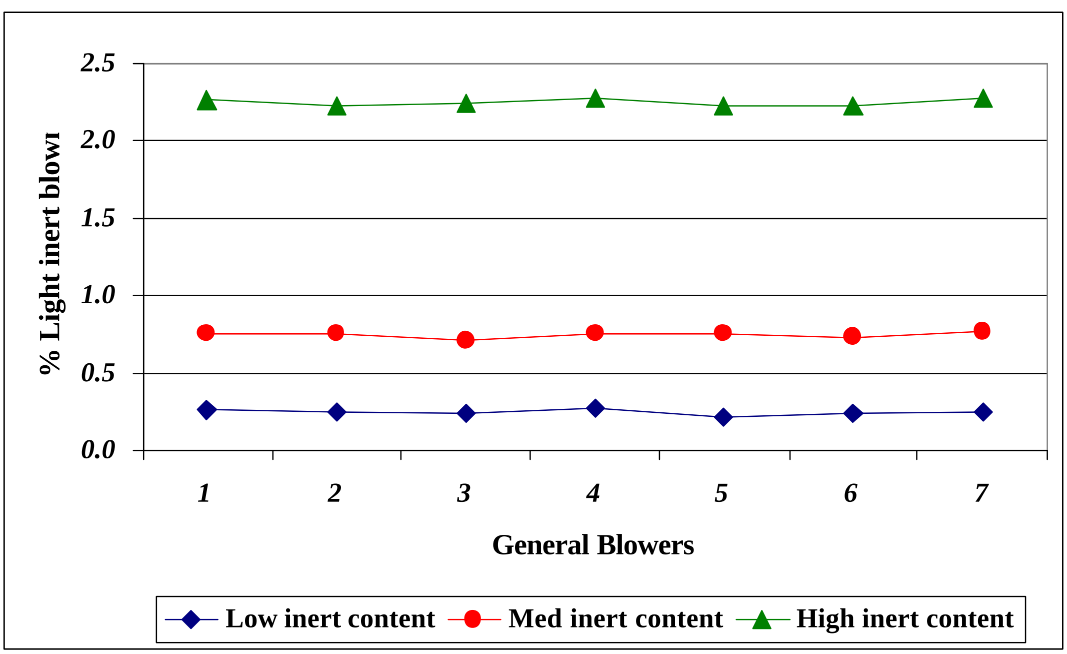
Uniformity of the TF uniform blowing procedure across blowers was tested using the protocol that was adopted by AOSA/SCST in June 2006. A tall fescue master calibration sample was used to calibrate seven General blowers at Oregon State University Seed Laboratory. Afterwards, the equivalent air velocity (EAV) value (m/s) for that point was determined for each blower using an anemometer (Model 271, Davis

Instruments, CA). The blowers were calibrated using one of the newly developed master calibration samples to determine the optimum blowing point for separating the inert matter from TF pure seed. The calibration was performed using the standard procedure described in Section 4.1 in the AOSA Handbook 24. The air velocity measurement was repeated twice to check for consistency. The study used three TF blind referee samples ranging from low to high amounts of material in the light portion The same blind samples were used in the seven blowers. Each sample was blown for three minutes using the EAV value determined above. The weight of the material in the light portion per gram to the 4th decimal places was recorded. The sample was blown four times in each blower.

Comparable results for all blind samples were achieved (Fig. 9) regardless of whether the sample contains high levels or low levels of light-weight inert matter. Two principles made it possible to achieve such uniform results across the seven blowers and across the three samples: 1) the use of a uniform master calibration sample to determine the optimum blowing point for all blowers; and 2) the use of equivalent air velocity value to reproduce the blowing point to test samples. These results strongly suggest again the value of a small but uniform number of "master" calibration samples to calibrate all blowers for achieving consistent results.

The above studies confirmed that a uniform blowing procedure in tall fescue is feasible. A national referee study is being planned to measure the repeatability of the proposed method across laboratories.

Fig. 9. Percentage of light inert separated from three tall fescue seed samples with different initial inert matter content (0.3-2.3%) using master calibration sample using seven blowers.



The application of this method would require:

a) General blowers, which are already available in most laboratories.

b) A master calibration sample of tall fescue to determine the optimum blowing point. This would be made available by AOSA/USDA Seed Testing Branch.

c) Anemometer (Model 271, Davis Instruments, CA) to measure the equivalent air velocity (m/sec) of blowing points. The anemometer is inexpensive and some labs already have one.

d) The equivalent air velocity value would be used as a standard calibration point to set the blower for daily testing work.

e) If the blower changes or repaired (i.e., new blower or new motor, belt, tube, etc) it would need to be recalibrated using a master calibration sample.

Conclusion and benefits for seed testing laboratories:

All the supporting evidence demonstrates that it is feasible to use a uniform blowing procedure to separate light-weight inert matter (of no planting value) from pure seeds in tall fescue samples. Once the blower is calibrated and the equivalent air velocity value of the optimum blowing point is determined, consistent blowing results can be achieved.

The supporting evidence also demonstrates it is not necessary to dismember the MSU to achieve an adequate separation of non-viable material. The proposed method will provide a more accurate assessment of the planting value of the seed unit structures as they are packaged for the marketplace.

This method eliminates the subjective and time-consuming visual/manual separation. It is inexpensive, simple, efficient, repeatable and finally it should increase uniformity in test results within and among laboratories.

If the proposal were adopted, the set of “master” calibration samples (approximately nine), would be made available to the AOSA/USDA to loan to interested laboratories. The “master” calibration samples would be returned to the same source to verify uniformity before loaning to other users. This method would assure uniformity of blowing points among all user laboratories.

REFERENCES

AOSA Rules for Testing Seeds. 2005. Association of Official Seed Analysts. Stillwater, OK.

AOSA Handbook Contribution No. 24 “Uniform Blowing Procedures”. Association of Official Seed Analysts. Stillwater, OK

SUBMITTED BY:
Adriel Garay, adriel.garay@oscs.orst.edu; Oregon State University Seed Laboratory 541-737-4794
Sabry Elias, sabry.elias@oscs.orst.edu; Oregon State University Seed Laboratory 541-373-4799
Deborah Meyer, dmeyer@cdfa.ca.gov, CA Dept. Food & Ag., the Seed Laboratory 916-262-1137

DATE SUBMITTED: October 15, 2006