

Rules Change Proposal – 15

PURPOSE OF PROPOSAL: To fine-tune the current blowing method of blue grama (*Bouteloua gracilis*), keeping some of the efficiencies it offers, but to classify florets with caryopses in the light portion as pure seed, and in some cases remove empty florets from the heavy portion and classifying them as inert.

The seed industry has been indicating that the purity results on blue grama obtained with the seed blower are usually lower than when the hand method was used. Statements have been made that seed growers have been losing millions of dollars each year since the hand method was discontinued in 1977. It took much longer to complete a purity test using the hand method, causing sample backlogs in seed labs. The blowing method greatly sped up the purity process and increased consistency in test results between labs. However it has been found that accuracy did suffer since dropping the hand method (see “Separating Pure Seeds from Blue Grama Using Three Purity Methods” at the end of the supporting evidence section of this proposal). Therefore, the objective of this proposal is to fine-tune the current blowing method of blue grama to increase accuracy and keep efficiency.

Present Rule:

Excerpts from the AOSA Rules for Testing Seeds Vol. 1:

Excerpt from table 2A:

Pure Seed Unit #	Kind of seed	Minimum weight for purity analysis ^a	Minimum weight for noxious-weed seed or bulk examination	Approximate number of seeds per gram ^b	Approximate number of seeds per ounce ^c
		Grams	Grams	Number	Number
23	<i>Bouteloua gracilis</i> (Kunth) Lag. Ex Griffiths blue grama	2	20	1,595	45,275

Excerpt from Table 3A. Pure seed unit definitions:

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 3.6. Special consideration: *For <i>Bouteloua curtipendula</i> , in addition to the units described above, spikelet group that disarticulates as a unit with rachis and internode.

3.6 Uniform blowing procedure

- d. Procedures: For samples with one kind of seed, the size of the samples to be blown shall be the same as that for a purity test except for blue grama and side-oats grama, which shall be divided into four approximately equal parts prior to blowing. All seed kinds are to be blown for three minutes. After completing the blowing procedure, remove all weed and crop seeds from the light portion and add these to the weed or crop separation, as appropriate. The remainder of the light portion shall be considered inert matter. Remove all weed and crop seeds and other inert matter (stems, leaves, dirt) from the heavy portion and add these to the weed, crop or inert

matter separations, as appropriate. The remainder of the heavy portion shall be considered pure seed.

- (6) Blue grama: The equivalent air velocity value (m/s) for blue grama shall be used. To determine this value, first determine the optimum calibration point for Kentucky bluegrass using a standard calibration sample. The blower gate opening value for the optimum calibration point shall be multiplied by a factor of 1.157 to obtain the adjusted gate opening value for blue grama. The factor of 1.157 is restricted to the General-type seed blower, see sections 3.3 and 7.2 in AOSA Rules for Testing Seeds Vol. 2. The blower gate shall be opened to the adjusted value and the equivalent air velocity value (m/s) shall be determined for blue grama. Before blowing, extraneous material that will interfere with the blowing process should be removed. The sample to be blown should be divided into four (4) approximately equal parts and each part blown separately.

Refer to AOSA Rules for Testing Seeds Vol. 2. for required additional procedures to prevent bunching of the seeds during the blowing procedure.

Excerpts from the AOSA Rules for Testing Seeds Vol. 2, Uniform Blowing Procedure: Excerpt from the Introduction (fifth paragraph):

Arnold L. Larsen, who chaired the Rangelgrass Analysis Subcommittee, developed and prepared written instructions for the blowing of blue grama (*Bouteloua gracilis*) and side-oats grama (*Bouteloua curtipendula*) that now must be tested by the uniform blowing method as required by the AOSA rules. Dr. Larson has conducted some investigations into calibration procedures, which resulted in some new procedures that are incorporated into this handbook.

5.4 Blue grama (*Bouteloua gracilis*).

The equivalent air velocity value (m/s) for blue grama shall be used. To determine this value, first determine the optimum calibration point for Kentucky bluegrass using a standard calibration sample. The blower gate opening value for the optimum calibration point shall be multiplied by a factor of 1.157 to obtain the adjusted gate opening value for blue grama. The factor of 1.157 is restricted to the General-type Seed Blower; see sections 3.3 and 7.2 in AOSA Rules Vol. 2. The blower gate shall be opened to the adjusted value and the equivalent air velocity value (m/s) shall be determined for blue grama.

The sample should be examined to remove extraneous material before blowing, and the sample then divided into four approximately equal parts. Each part is blown separately, and the analyst should ensure that the seeds in the blower are freely moving as bunching can result in variable results. The resulting heavy fractions are combined, and the same is done for the light fractions.

6.6 Procedure for purity analysis of blue grama (*Bouteloua gracilis*)

STEP 1. Separating the light fraction.

The light fractions from the four blowings are combined, and the other crop seed, weed seed, and inert matter are separated according to Sections 3.2-3.8 of the AOSA Rules Vol. 1. The large extraneous matter, which was removed before the blowings, is added to the inert matter component of the sample.

All florets of blue grama blown into the light fraction are considered inert matter.

STEP 2. Separating the heavy fraction.

Other crop seeds, weed seeds, seed-like particles and inert matter (sticks, sand, etc.) are classified in accordance with Sections 3.2-3.8 of the AOSA Rules Vol. 1.

All blue grama seed units remaining in the heavy fraction are to be considered pure seed. However, broken florets or caryopses one-half or less the original size are considered inert matter. Seed units with fungus bodies, such as ergot are classified in accordance with Section 3.5 a of the AOSA Rules Vol. 1.

6.7. Procedure for purity analysis of side-oats grama (*Bouteloua curtipendula*).

The procedure given in Section 6.6 for blue grama is to be used for the purity analysis of side-oats grama.

PROPOSED RULE:

Excerpts, additions and changes to the AOSA Rules for Testing Seeds Vol. 1:

Excerpt from Table 2A (for review, no change proposed to Table 2A):

Pure Seed Unit #	Kind of seed	Minimum weight for purity analysis ^a	Minimum weight for noxious-weed seed or bulk examination	Approximate number of seeds per gram ^b	Approximate number of seeds per ounce ^c
		Grams	Grams	Number	Number
23	<i>Bouteloua gracilis</i> (Kunth) Lag. Ex Griffiths blue grama	2	20	1,595	45,275

Excerpt from Table 3A. Pure seed unit definitions:

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 3.6. Special considerations: <ul style="list-style-type: none"> For <i>Bouteloua curtipendula</i>, in addition to the units described above, spikelet group that disarticulates as a unit with rachis and internode. For <i>Bouteloua gracilis</i>, in addition to the units described above, see section 3.6d(6) for the classification of empty florets found in the heavy portion and florets containing caryopses at least 1/3 the length of the floret found in the light portion.

3.6 Uniform blowing procedure

- d. Procedures: For samples with one kind of seed, the size of the samples to be blown shall be the same as that for a purity test except for blue grama and side-oats grama, which shall be divided into four approximately equal parts prior to blowing. Before blowing, extraneous material (e.g., large stems and leaf fragments, soil, stones, other non-plant material, and seeds of other species that might entangle the kind being tested) that will interfere with the blowing process should be removed (refer to Sec. 6.2 of AOSA Rules for Testing Seeds Vol. 2). All seed kinds are to be blown for three minutes. After completing the blowing procedure, remove all weed and crop seeds from the light portion and add these to the weed or crop separation, as appropriate. The

remainder of the light portion shall be considered inert matter (see additional instructions in 3.6d(6) of AOSA Rules for Testing Seeds Vol. 1, for blue grama). Remove all weed and crop seeds and other inert matter (stems, leaves, dirt) from the heavy portion and add these to the weed, crop or inert matter separations, as appropriate. The remainder of the heavy portion shall be considered pure seed (see additional instructions in 3.6d(6) of AOSA Rules for Testing Seeds Vol. 1., for blue grama). Add any extraneous inert material removed prior to blowing to the inert matter portion. If seeds of other crops and weeds were removed prior to blowing, these shall be added to the appropriate category.

3.6d

(6) Blue grama: The equivalent air velocity value (m/s) for blue grama shall be used. To determine this value, first determine the optimum calibration point for Kentucky bluegrass using a standard calibration sample. The blower gate opening value for the optimum calibration point shall be multiplied by a factor of 1.157 to obtain the adjusted gate opening value for blue grama. The factor of 1.157 is restricted to the General-type seed blower, see sections 3.3 and 7.2 in AOSA Rules for Testing Seeds Vol. 2. The blower gate shall be opened to the adjusted value and the equivalent air velocity value (m/s) shall be determined for blue grama. Before blowing, ~~remove any extraneous~~ material that will interfere with the blowing process ~~should be removed~~. The sample to be blown ~~should~~ shall be divided into four (4) approximately equal parts and each part blown separately. Throughout the blowing procedure, watch the seeds in the seed cup of the blower, and carefully agitate the seed cup as the seeds begin to bunch up.

~~Refer to AOSA Rules for Testing Seeds Vol. 2. for required additional procedures to prevent bunching of the seeds during the blowing procedure.~~

STEP 1. Separating the light fraction.

The light fractions from the four blowings are combined, and the other crop seed, weed seed, and inert matter are separated according to Sections 3.2-3.8 of the AOSA Rules for Testing Seeds Vol. 1. Additionally, check the combined light portions for florets containing caryopses at least 1/3 the length of the floret. All such seed units shall be added to the pure seed. The large extraneous matter, which was removed before the blowings, is added to the inert matter component of the sample. All remaining empty florets of blue grama blown into the light fraction are considered inert matter.

STEP 2. Separating the heavy fraction.

The heavy fractions from the four blowings are combined, and other crop seeds, weed seeds, seed-like particles and inert matter (sticks, sand, etc.) are classified in accordance with Sections 3.2-3.8 of the AOSA Rules for Testing Seeds Vol. 1. Additionally, thoroughly mix the combined heavy portions, count 100 units of the heavy portion, and check for empty florets by use of magnification and light pressure. If five (5) or more empty florets are found in the 100 seed check, the entire heavy portion shall be examined, and all empty florets shall be removed and added to the inert matter. If four (4) or fewer empty florets are found in the 100 seed check, no further examination is required for empty florets, and the empty florets found in the 100 seed check shall to be added to the inert matter. All blue grama seed units remaining in the heavy fraction are to be considered pure seed. However, broken florets or caryopses one-half or

less the original size are considered inert matter. Seed units with fungus bodies, such as ergot are classified in accordance with Section 3.5a of the AOSA Rules for Testing Seeds Vol. 1.

Excerpts, additions and changes to the AOSA Rules for Testing Seeds Vol. 2, Uniform Blowing Procedure:

Excerpt, changes and additions to the Introduction (fifth paragraph):

Arnold L. Larsen, who chaired the Rangeland Analysis Subcommittee, developed and prepared written instructions for the blowing of blue grama (*Bouteloua gracilis*) and side-oats grama (*Bouteloua curtipendula*) ~~that now must~~ to be tested by the uniform blowing method as required by the AOSA rules. Dr. Larson has conducted some investigations into calibration procedures, which resulted in some new procedures that are incorporated into this handbook.

New last paragraph added to Introduction:

Gil Waibel added modifications to the blowing procedure methods of determining pure seed and inert matter of blue grama in 2010. This method maintains the use of the blowing point for blue grama, but the light portion must be examined for florets containing caryopses that are at least one-third the length of the floret. These florets are classified as pure seed. A fraction (100 seeds) of the heavy portion is checked for empty florets and in some cases these are removed and classified as inert matter.

5.4 Blue grama (*Bouteloua gracilis*).

The equivalent air velocity value (m/s) for blue grama shall be used. To determine this value, first determine the optimum calibration point for Kentucky bluegrass using a standard calibration sample. The blower gate opening value for the optimum calibration point shall be multiplied by a factor of 1.157 to obtain the adjusted gate opening value for blue grama. The factor of 1.157 is restricted to the General-type Seed Blower; see sections 3.3 and 7.2 in AOSA Rules Vol. 2. The blower gate shall be opened to the adjusted value and the equivalent air velocity value (m/s) shall be determined for blue grama.

The sample ~~should~~ shall be examined to remove extraneous material before blowing, and the sample then divided into four approximately equal parts. Each part is blown separately, and the analyst ~~should~~ shall ensure by careful seed cup agitation that the seeds in the blower are freely moving as bunching can result in variable results. The resulting heavy fractions are combined, and the same is done for the light fractions as instructed in section 6.6 of this volume.

6.6 Procedure for purity analysis of blue grama (*Bouteloua gracilis*)

STEP 1. Separating the light fraction.

The light fractions from the four blowings are combined, and the other crop seed, weed seed, and inert matter are separated according to Sections 3.2-3.8 of the AOSA Rules Vol.1. Additionally, check the combined light portions for florets containing caryopses at least 1/3 the length of the floret. All such seed units shall be added to the pure seed. The large extraneous matter, which was removed before the blowings, is added to the inert matter component of the sample.

All remaining empty florets of blue grama blown into the light fraction are considered inert matter.

STEP 2. Separating the heavy fraction.

The heavy fractions from the four blowings are combined, and other crop seeds, weed seeds, seed-like particles and inert matter (sticks, sand, etc.) are classified in accordance with Sections 3.2-3.8 of the AOSA Rules Vol. 1.

Additionally, thoroughly mix the combined heavy portions, count 100 units of the heavy portion, and check for empty florets by use of magnification and light pressure. If five (5) or more empty florets are found in the 100 seed check, the entire heavy portion shall be examined, and all empty florets shall be removed and added to the inert matter. If four (4) or fewer empty florets are found in the 100 seed check, no further examination is required for empty florets, and the empty florets found in the 100 seed check shall be added to the inert matter. All blue grama seed units remaining in the heavy fraction are to be considered pure seed. However, broken florets or caryopses one-half or less the original size are considered inert matter. Seed units with fungus bodies, such as ergot are classified in accordance with Section 3.5 a of the AOSA Rules Vol. 1.

6.7. Procedure for purity analysis of side-oats grama (*Bouteloua curtipendula*).

~~The procedure given in Section 6.6 of blue grama is to be used for the purity analysis of side-oats grama.~~

STEP 1. Separating the light fraction.

The light fractions from the four blowings are combined, and the other crop seed, weed seed, and inert matter are separated according to Sections 3.2-3.8 of the AOSA Rules Vol. 1. The large extraneous matter, which was removed before the blowings, is added to the inert matter component of the sample. All florets of side-oats grama blown into the light fraction are considered inert matter.

STEP 2. Separating the heavy fraction.

Other crop seeds, weed seeds, seed-like particles and inert matter (sticks, sand, etc.) are classified in accordance with Sections 3.2-3.8 of the AOSA Rules Vol. 1.

All side-oats grama seed units remaining in the heavy fraction are to be considered pure seed. However, broken florets or caryopses one-half or less the original size are considered inert matter. Seed units with fungus bodies, such as ergot are classified in accordance with Section 3.5a of the AOSA Rules Vol. 1.

HARMONIZATION AND IMPACT STATEMENT:

Federal Seed Act: The language in the Federal Seed Act concerning the use of the blowing point for blue grama is similar to the AOSA rule as it currently stands. If this proposal passes, there will be a difference in procedures between the Federal Seed Act and the AOSA Rules.

Although the two methods will be different, the net result for the proposed AOSA method is a higher percentage of pure seed, and a lower percentage of inert matter. Using the standard one-way tolerance for regulatory purposes there could be violations if the Federal Seed Act method is used to check a label created using this proposed AOSA method. Conversely, there may be no violation if the proposed AOSA method is used to test a label created using the FSA method. This issue has been discussed with members of the seed industry who

produce and sell blue grama, and they are willing to suffer the consequences until, and if, the Federal Seed Act is updated to be equivalent to this proposed AOSA method.

Canadian Methods and Procedures: The M&P does not list blue grama, so there is no conflict.

ISTA: There is currently a conflict with the ISTA rules. The ISTA pure seed definition for blue grama is “no need to check for the presence of a caryopsis.” ISTA does not use a blowing point for blue grama. The ISTA rule for blue grama is much like the “modified” AOSA rule between 1977 and 1981. This rule had a severe impact on the U.S. seed industry, and did not last long in the AOSA Rules.

It should be noted that almost all seed lots of blue grama are produced and sold in the United States. There are a few seed lots sold in Mexico each year. Almost all blue grama is tested by the AOSA Rules and the Federal Seed Act, and very little blue grama is tested by the ISTA method.

SUPPORTING EVIDENCE:

There are two supporting studies attached to this proposal. The first is a referee, and the second is a research study comparing three purity methods on blue grama.

AUTHOR’S NOTE: Keeping the blowing point intact will give all labs an equal starting point before hand picking the light portion, and in some cases both the light and the heavy portions. This method will improve the uniformity of the hand-picking method, because of a set blowing separation, and usually only needing to hand pick the light portion, which is generally much less seed than in the heavy portion. In the study “Separating Pure Seeds from Blue Grama Using Three Purity Methods” in the supporting evidence section of this proposal, only one seed lot of the ten lots tested would have required hand picking both the light and heavy portions. By keeping the blowing point intact, there will be time savings using the blowing point supplemented with the proposed hand picking.

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DATE SUBMITTED: October 15, 2009; revised January 25, 2010

Referee:

Title: Determination of the uniformity between labs of a modified Blue Grama blowing procedure.

Purpose: To compare the results of 8 seed laboratories to see if a modified blowing procedure of blue grama gives acceptable uniform results.

Materials and Methods:

Participating labs were: Arkansas Valley Seed Solutions Lab, Iowa State University Seed Lab, Colorado Seed Lab, Lubbock Seed Lab, Ohio Seed Improvement Association Lab, South Dakota State University Seed Lab, Sterling Seed Testing, and the Wyoming Seed Analysis Lab.

Three samples of differing purity levels were used. The samples were divided at the Wyoming Seed Analysis Lab to assure uniformity in the dividing procedure. The working weight of each replicate was 0.5 grams, which is ¼ of an official purity sample for blue grama. Each lab tested four 0.5 gram samples.

General seed blower, anemometer and scales capable of weighing seeds to a thousandth of a gram (0.001 gram) were used in the blowing procedures and purity analyses.

Purity Procedure:

All extraneous material was removed from the sample before each blowing.

Blower setting was checked before each blowing with an anemometer.

Sample placed in seed cup of the blower, and blown for three minutes. During the entire blowing period, the sample in the seed cup was watched for bunching, and the seed cup was carefully agitated as bunching occurred.

Light portion was hand-picked, checking if there were any florets with caryopses at least 1/3 the length of the floret. These florets were added to the pure seed portion of the sample. All other crop and weed seeds were removed as appropriate.

The heavy portion had a 100 seed check for empty florets. If 5 or more empty florets were found, the entire heavy portion was hand-picked. If 4 or fewer empty florets were found, the sample was not hand-picked. All other crop, weed seed, and inert matter (other than empty florets) were removed as appropriate.

Purity percentages were calculated, and returned to the Wyoming Seed Analysis Lab for compilation and analysis.

Results and Discussion:

Significant differences were found among seed labs for purity percentage on samples 1 and 2 in this study (Tables 1 and 3).

Table 1: Analysis of Variance for Sample 1

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	Prob
Replication	3	3.312	1.104	0.4558	
Lab	7	100.727	14.39	5.9400	0.0007
Error	21	50.872	2.422		
Total	31	154.911			

Table 2: Mean Comparisons Among Purity Percentage for Eight Labs on Sample 1

	Rep 1	Rep 2	Rep 3	Rep 4	Average	Significance Rank
Lab 1	82.95	85.11	84.18	82.52	83.69	AB
Lab 2	80.39	79.96	82.70	81.46	81.13	C
Lab 3	86.52	85.62	83.04	86.03	85.30	A
Lab 4	85.53	82.00	83.53	87.75	84.75	A
Lab 5	81.21	82.73	79.03	78.76	80.43	C
Lab 6	85.39	84.90	84.40	84.70	84.85	A
Lab 7	84.91	84.91	84.52	83.07	84.35	A
Lab 8	82.09	83.15	81.77	79.72	81.68	BC
				Mean	83.27	
				Max	85.30	
				Min	80.43	
				Max-Min	4.87	

Coefficient of Variation: 1.87%

Least Significant Difference Test: LSD Value = 2.289 at alpha = 0.05

Five labs show no significant differences between purity percentages (designated by the letter “A” in the right column of Table 2). Lab 8 was not significantly different than Lab 1, but was significantly different than Lab 3, 4, 6 and 7. Two labs ranked “C” were significantly different than all other labs except Lab 8.

Table 3: Analysis of Variance for Sample 2

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	Prob
Replication	3	14.748	4.916	2.6822	0.0730
Lab	7	70.360	10.051	5.4840	0.0011
Error	21	38.490	1.833		
Total	31	123.598			

Table 4: Mean Comparisons Among Purity Percentage for Eight Labs on Sample 2

	Rep 1	Rep 2	Rep 3	Rep 4	Average	Significance Rank
Lab 1	77.14	76.95	77.57	77.51	77.29	AB
Lab 2	78.50	74.56	74.66	73.50	75.31	BC
Lab 3	77.37	75.85	77.12	78.39	77.18	AB
Lab 4	77.11	79.53	78.33	75.38	77.59	A
Lab 5	77.25	72.94	75.43	73.85	74.87	C
Lab 6	76.98	74.74	77.25	75.06	76.01	ABC
Lab 7	77.99	74.28	77.29	75.10	76.17	ABC
Lab 8	72.03	73.19	72.90	73.05	72.79	D
				Mean	75.90	
				Max	77.59	
				Min	72.79	
				Max-Min	4.80	

Coefficient of Variation: 1.78%

Least Significant Difference Test: LSD Value = 1.991 at alpha = 0.05

Similar to sample 1, the same five labs showed no significant differences between purity percentages with sample 2 (Table 4). Lab 5 was significantly different than Labs 1, 3, 4 and 8. Lab 8 was significantly different than all other labs.

Table 5: Analysis of Variance on Sample 3

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	Prob
Replication	3	0.856	0.285	2.2736	0.1096
Lab	7	0.948	0.135	1.0795	0.4105
Error	21	2.634	0.125		
Total	31	4.437			

Table 6: Mean Comparisons Among Purity Percentage for Eight Labs on Sample 3

	Rep 1	Rep 2	Rep 3	Rep 4	Average	
Lab 1	99.93	99.40	99.33	99.68	99.59	A
Lab 2	99.23	98.14	99.39	99.93	99.17	A
Lab 3	99.85	99.67	99.84	99.38	99.69	A
Lab 4	99.23	99.48	99.02	99.57	99.33	A
Lab 5	99.48	99.15	98.96	99.31	99.23	A
Lab 6	99.55	99.70	99.13	99.77	99.54	A
Lab 7	99.34	99.07	99.14	99.60	99.29	A
Lab 8	99.27	99.84	98.86	99.83	99.45	A
				Mean	99.41	
				Max	99.69	
				Min	99.17	
				Max-Min	0.52	

Coefficient of Variation: 0.36%

Least Significant Difference Test: LSD Value = N/S at alpha = 0.05

There were no significant differences between the 8 participating labs for sample 3 (Table 6).

The uniformity of tests between labs is not as high as might be expected on most grass species. Samples 1 and 2 had the same 5 out of 8 labs showing no significant differences (Tables 2 and 4). Sample 3 had all 8 labs showing no significant differences (Table 6). The first two samples had lower pure seed percentages than the high percentage for sample 3. It would be expected that sample three have a higher repeatability between labs, due to its more uniform character.

In 1981, the rules proposal introducing the blowing point for blue grama, showed the following results in Table 7:

Table 7: Purity Percentages from 1981 Blue Grama Blowing Point Referee
(AOSA Feb. 1981 Newsletter, page 21-24)

	Lot 1	Lot 3	Lot 4
Lab 1	30.05	32.72	79.15
Lab 2	32.43	36.72	80.92
Lab 3	35.59	35.93	81.94
Lab 4	36.22	36.81	82.70
Lab 5	35.59	34.33	80.50
Lab 6	28.22	35.27	83.05
Lab 7	28.58	31.39	80.55
Lab 8	32.54	37.38	80.78
Lab 9	34.06	36.50	78.98
Lab 10	30.32	24.75	80.60
Mean	32.36	34.18	80.92
Max	36.22	37.38	83.05
Min	28.22	24.75	78.98
Max-Min	8.00	12.63	4.07

Note: There was no Lot 2 in the referee.

The results from this referee and the referee in 1981 both suggest that blue grama has inherent uniformity issues. The difference between the maximum purity and the minimum purity percentages in this referee was Sample 1 (4.87% in Table 2), Sample 2 (4.80% in Table 4), and Sample 3 (0.52% in Table 6). The 1981 referee had differences between the maximum purity and minimum purity percentages for Lot 1 (8.00%), Lot 3 (12.63%), and Lot 4 (4.07%) in Table 7. The presence of high levels of chaff, stems, stones and dirt balls, and many empty florets cause blue grama to have more than normal variability between labs, and even tests. Different general seed blowers, and how they were calibrated might also contribute to the differences. Statistical analysis of the 1981 data is not possible, because the replicate data was not published, and is no longer in existence. However, these data for blue grama indicates higher than normal variability between labs. Thus the results shown in this referee are not unexpected.

A second unconventional statistical approach was used to analyze the data in this referee. The purity percentage from each lab was checked to see if it was in tolerance with the mean from all labs in the referee on each seed sample. The AOSA tolerance Table 13A was used to determine if the test result from each lab was in tolerance with the mean found in the referee.

Table 8: Determining if Purity Percentage for Each Lab is in Tolerance With the Referee Mean on Sample 1

	Purity Average %	Tolerance w/mean (Avg.+Mean)/2	Avg.-Mean Difference	AOSA Tolerance	Within Tolerance
Lab1	83.69	83.48	0.42	2.76	Yes
Lab 2	81.13	82.20	-2.14	2.76	Yes
Lab 3	85.30	84.29	2.03	2.62	Yes
Lab 4	84.70	83.99	1.43	2.76	Yes
Lab 5	80.43	81.85	-2.84	2.88	Yes
Lab 6	84.85	84.06	1.58	2.62	Yes
Lab 7	84.35	83.81	1.08	2.76	Yes
Lab 8	81.68	82.48	-1.59	2.76	Yes
Mean	83.27				

The purity results in Table 8 from all labs on sample 1 were in tolerance with the mean.

Table 9: Determining if Purity Percentage for Each Lab is in Tolerance With the Referee Mean on Sample 2

	Purity Average %	Tolerance w/mean (Avg.+Mean)/2	Avg.-Mean Difference	AOSA Tolerance	Within Tolerance
Lab1	77.29	76.60	1.39	3.09	Yes
Lab 2	75.31	75.61	-0.59	3.18	Yes
Lab 3	77.18	76.54	1.28	3.09	Yes
Lab 4	77.59	76.75	1.69	3.09	Yes
Lab 5	74.87	75.39	-1.03	3.18	Yes
Lab 6	76.01	75.96	0.11	3.18	Yes
Lab 7	76.17	76.04	0.27	3.09	Yes
Lab 8	72.79	74.35	-3.11	3.18	Yes
Mean	75.90				

The purity results in Table 9 from all labs on sample 2 were in tolerance with the mean.

Table 10: Determining if Purity Percentage for Each Lab is in Tolerance With the Referee Mean on Sample 3

	Purity Average %	Tolerance w/mean (Avg.+Mean)/2	Avg.-Mean Difference	AOSA Tolerance	Within Tolerance
Lab1	99.59	99.50	0.18	0.54	Yes
Lab 2	99.17	99.29	-0.24	0.67	Yes
Lab 3	99.69	99.55	0.28	0.52	Yes
Lab 4	99.33	99.37	-0.08	0.63	Yes
Lab 5	99.23	99.32	-0.18	0.63	Yes
Lab 6	99.54	99.48	0.13	0.58	Yes
Lab 7	99.29	99.35	-0.12	0.63	Yes
Lab 8	99.45	99.43	0.04	0.58	Yes
Mean	99.41				

The purity results in Table 10 from all labs on sample 3 were in tolerance with the mean.

When comparing the result of each lab with the mean, and using the AOSA Tolerance table 13A, the purity results of all labs on all three samples were in tolerance with the sample mean.

Conclusion:

Uniformity between labs had some significant differences. It is helpful to see the results from the referee conducted in 1981 used as justification to establish a blowing point for blue grama. Similar results concerning lab uniformity were obtained in this referee and that of 1981. Blue grama is not uniform by nature, and there are many variables that can cause less than desirable uniformity between labs. It is reasonable to conclude that the results of this referee give as much uniformity between labs as is currently possible.

Separating Pure Seeds from Blue Grama (*Bouteloua gracilis*) Using Three Purity Methods

Background of the problem:

The original official purity technique to determine pure seed in blue grama was to use the hand method. According to A.L. Larson and L. E. Weisner this was a very time consuming method, and results were not consistent between labs.^{1,2} There have been questions by seed analysts and industry members since the blowing point became part of the AOSA rules in 1981, about the accuracy of the official blowing method. “Pure seed” (florets with caryopses), in differing amounts, are being blown into the light portion of the blowings.

In 1977 a new rule was proposed and passed to use a “modified method” of testing blue grama.^{2,3} The “modified method” proposed by L.E. Wiesner, chair of the rules committee, removed technical variability by making all florets (florets that have a caryopses and all empty florets.) pure seed.² Wiesner stated that the modified method was designed to make the purity results more consistent. R. Danielson, chair of the Rules Committee, stated in 1981, that the pure live seed (PLS) decreased with the “modified method”, and the industry was complaining.⁴ In 1981, the blowing procedure rule proposal for blue grama was introduced by the Rules Committee chair R. Danielson.⁴ The Rangelgrass Committee chair K. Boatwright published a referee comparing three different purity methods including the blowing point for blue grama, and also recommended using the blowing point procedure.⁵ The blowing point procedure for blue grama became the official method in the AOSA rules in 1981.⁷

The blowing method brought more consistency in purity results, but some accuracy was lost. Industry has claimed that they have been losing millions of dollars with the blowing point, and would like a more accurate method developed. It is interesting that the rule proposal introducing the blowing point for blue grama in 1981 indicated “It appears that the blowing method would be a reasonable compromise between the high reproducibility and time savings achieved by the modified method and the analytical values produced by the hand method.”⁴ In 1981 in a Rangelgrass Committee report, chair K. Boatwright stated: “The time saving factor would insure a quicker return of analysis results to the seedsmen and also prevent work backlogs in the seed industry.”⁵ Industry members are willing to pay the additional costs needed to test blue grama, because of lost revenue due to the mechanically arbitrary way the seed blower sends “pure seeds” into the light portion in differing amounts from seed lot to seed lot.

Rationale:

Time savings and backlogs are important, and timely results are critical during certain seasons of the year. The AOSA rules also challenge us to “in all cases the ultimate purpose of making a test is to determine the value of the seed for planting.” This study will determine if the blowing method was both consistent and accurate compared to the hand method. The purity percentage was determined on ten seed lots using the following methods:

1. Official method using the blowing point (Official Method “OM”)
2. Picking florets with a caryopses at least 1/3 the length of the floret and including the weight of these florets with the pure seed. (Pick light “PL”)
3. Pick the florets from the light portion as in number two above, and include the weight of all empty florets found in the heavy portion with the inert matter. (Pick both “PB”)

Objective:

This study will evaluate the consistency and accuracy of the purity result of the official blowing method. The objective of this study is to determine:

1. If “pure seeds” are blowing over into the light portion;
2. If empty florets remain in the heavy portion;

3. If the weight of the florets with caryopses in the light portion cancel out the weight of the empty florets in the heavy portion;
4. If there are significant differences between the hand-pick method and the blowing method;
5. If there is a way to fine-tune the blowing method to be more accurate.

Materials and Methods:

Seed sources:

Samples from ten seed lots were selected from various sources: varieties (5 varieties which includes native collected), area of production (5 states), year of production (1 from 2006, and 3 each from production years 2006, 2007, and 2008), irrigated (8 yes, 2 no), and certified (4 yes, and 6 no).

Seed Source Table

Sample	Variety	Origin	Year Produced	Irrigated	Certified
1	Alma	Texas	2008	Yes	No
2	Hachita	Texas	2006	Yes	No
3	Bad River	Minnesota	2008	Yes	No
4	Alma	Colorado	2005	Yes	Yes
5	Alma	Colorado	2006	Yes	Yes
6	Hachita	Colorado	2007	Yes	Yes
7	Native(Collected)	New Mexico	2006	No	No
8	Lovington	Colorado	2007	Yes	Yes
9	Birds-eye	Wyoming	2007	Yes	No
10	Native(Collected)	New Mexico	2008	No	No

Other Materials:

General Seed Blower

Anemometer

Microscope used over purity board

Fine #3 titanium forceps

Balance capable of weighing up to ten thousandths of a gram (0.0001 gram)

Method of purity analysis:

1. Four replicates of 0.5000+ grams were individually divided by the official table top method and tested for each seed sample
2. Stems were removed before using blowing procedure
3. Blower was checked with anemometer before each blowing
4. Replicate was blown at official setting for blue grama
5. Remaining inert and other species if any were removed from the heavy portion and the “official” purity was calculated for each replicate (an official purity is based on all four replicates combined together, where for the purposes of this study, purity percentages were calculated on 1/4 of the official amount for a purity test)
6. Light portion was examined for florets containing caryopses at least 1/3 the length the floret, which were removed and weighed (to be added to the weight of the heavy portion)
7. Heavy portion was examined and all empty florets were removed and weighed to be added to the total inert (for the both picked method)

Once the procedure described above was completed, the following purity percentages were calculated:

1. Official AOSA method (OM).
2. Official method + Pick pure seed from light fraction (PL).
3. Official method + Pick both fractions (PB): pure seed from the light fraction and inert matter from the heavy fractions.

Experimental design: Completely Randomized Design with two factors: factor A, samples and factor B, purity methods, with four replications.

Data was subjected to analysis of variance. Means were separated using LSD. MSTAT statistical package was used to analyze the data.

Results and Discussion:

The ANOVA indicated that both samples and method of separating pure seeds from blue grama had significant effect of the percentage of pure seed results (Table 1).

The pure seeds separated from the ten samples ranged between 48.57% and 99.48% using the official method (OM)(Table 2). This reflects the effect of seed production practices, weather conditions, variety, time of harvest, and level of seed cleaning on the final purity of a sample.

Table 1: Analysis of variance for factors affecting purity analysis of ten samples of blue grama tested by three methods.

	Degrees of	Sum of	Mean	F	
Source	Freedom	Squares	Squares	Value	Prob
Samples	9	22776.034	2530.670	217.6029	0.0000
Error	30	348.893	11.630		
Purity method	2	268.474	134.237	168.1349	0.0000
AB	18	181.852	10.103	12.6541	0.0000
Error	60	47.903	0.798		
Total	119	23623.156			

Coefficient of Variation: 1.24%

The highest pure seed content was obtained when the current method + pick pure seed from light fraction (PL) was used; the lowest when the official method (OM) was used and the current method + pick both fractions (PB): pure seed from the light fraction and inert matter from the heavy fractions was in between (Table 2 and Fig. 1).

The pure seed contents of five out of the ten samples used in the study were within tolerance whether the official method (OM) or the official method + pick pure seed from light fraction (PL) was used (Table 2).

Table 2: Determination if Methods for Picking the Light Portion (PL) and Picking Both Light and Heavy Portions (PB) are in Tolerance with the Results of the Official Method (OM)

Sample #	Percent of pure seeds evaluated by three methods			Difference between methods		Mean of (PL+OM)/2	AOSA Rules Table 13A tolerances for both means of: (PL+OM)/2 and (PB+OM)/2	PL-OM Within Tolerance	Mean (PB+OM)/2	PB-OM Within Tolerance
	Official AOSA method (OM)	Pick pure seed from light portion (PL)	Pick both light and heavy portions (PB)	PL-OM	PB-OM					
	%	%	%							
1	65.30	68.36	67.83	3.06	2.53	66.83	3.44	Yes	66.57	Yes
2	55.29	63.11	62.67	7.82	7.38	59.20	3.65	No	58.98	No
3	99.48	99.55	99.55	0.07	0.07	99.52	0.54	Yes	99.52	Yes
4	71.57	78.42	78.16	6.85	6.59	75.00	3.18	No	74.87	No
5	48.57	53.15	52.84	4.58	4.27	50.86	3.65	No	50.71	No
6	78.38	80.45	79.69	2.07	1.31	79.42	2.99	Yes	79.04	Yes
7	72.12	73.61	73.06	1.49	0.94	72.87	3.26	Yes	72.59	Yes
8	64.92	67.68	66.13	2.76	1.21	66.30	3.44	Yes	65.53	Yes
9	87.71	88.10	86.43	0.39	-1.28	87.91	2.47	Yes	87.07	Yes
10	53.98	58.93	58.63	4.95	4.65	56.46	3.65	No	56.31	No
Mean	69.73	73.14	72.50	3.40	2.77	71.44	3.33	No	71.12	Yes
Max	99.48	99.55	99.55	7.82	7.38					
Min	48.57	53.15	52.84	0.07	-1.28					
Max-Min	50.91	46.40	46.71	7.75	8.66					
SD	15.78	13.98	14.10	2.61	2.85					

Figure 1: Comparative Chart of Purity Percentages of Three Methods on Ten Blue Grama Samples

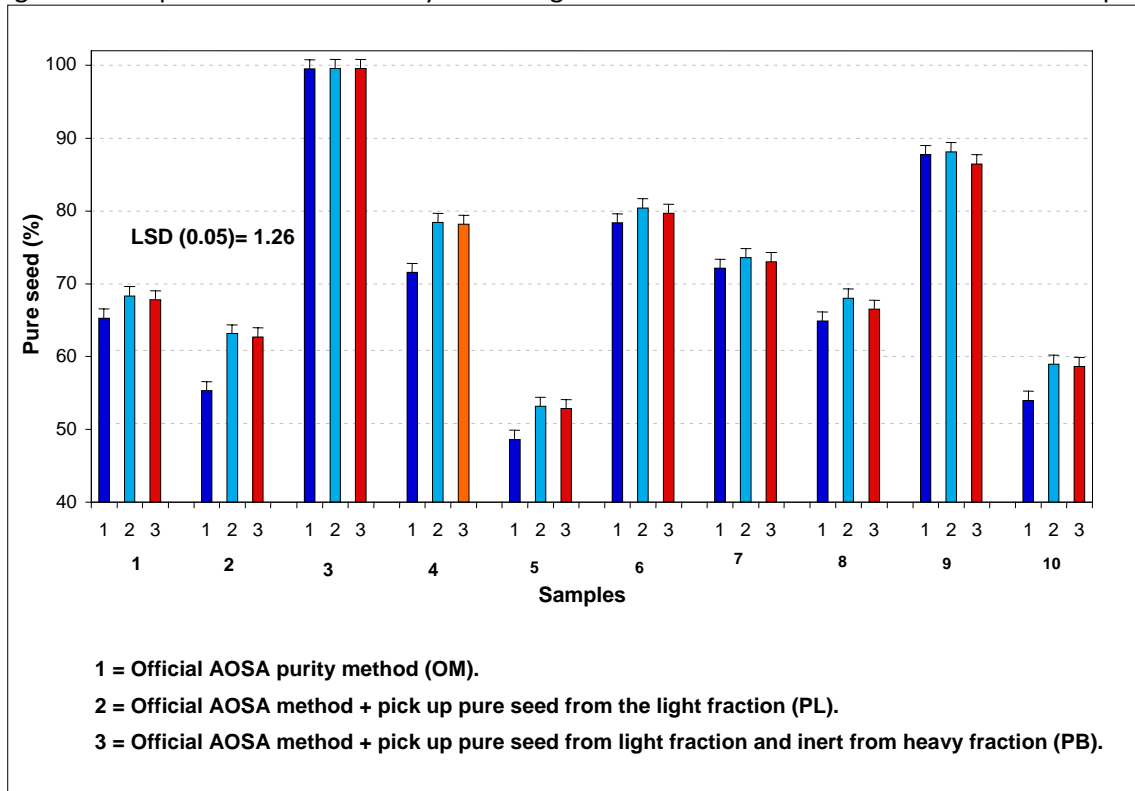


Table 3: Four Replicate Total of Blue Grama Units Removed from the Light and Heavy Portions of the Blowing Procedure

Sample	Light Portion		Heavy Portion		Net Weight (gms)
	# Florets	Weight (gms)	# Empty florets	Weight (gms)	
1	124	0.0648	36	0.0112	0.0536
2	352	0.1665	33	0.0095	0.1570
3	4	0.0015	0	0.0000	0.0015
4	201	0.1130	17	0.0054	0.1076
5	192	0.0929	18	0.0062	0.0867
6	93	0.0437	48	0.0160	0.0277
7	74	0.0303	32	0.0112	0.0191
8	124	0.0694	85	0.0317	0.0377
9	20	0.0219	119	0.0358	-0.0139
10	229	0.1073	21	0.0065	0.1008

Every sample had florets with caryopses in the light portion (ranged from 4 to 352 florets). Only one sample had no empty florets in the heavy portion. There was much more impact on the purity percentage from the florets removed from the light portion, than the empty florets removed from the heavy portion. One sample (#9) had more empty florets in the heavy portion than florets in the light portion, and this impact would lower the purity compared to the official method.

Table 4: Total Viability of the Ten Seed Samples in this Study, Based on the Official (OM), Pick Light (PL) and Pick Both (PB) Methods

Sample #	Total Viable Official Method (OM)	Total Viable Pick Light (PL)	Total Viable Pick Both (PB)
1	88	87	89
2	87	86	87
3	96	96	96
4	89	88	89
5	92	92	93
6	89	89	91
7	56	56	57
8	81	80	84
9	86	86	90
10	92	91	92
Mean	86	85	87

This table is included to help calculate the pure live seed (PLS) percentage in table 5 to help illustrate the financial impact the official method has on the seed industry by blowing florets with caryopses into the light portion.

The data in this study presents a maximum difference of 7.82% between the official method (OM) and the pick light method (PL) (Table 2). There is a maximum difference of 7.38% between the official method (OM) and the pick both light and heavy portions method (PB) (Table 2). When comparing both the pick light (PL) and the pick both methods (PB) with the official method (OM), four of the 10 lots were out of tolerance (Table 2). When considering the 3.40 difference between the official method and the pick light method, and the 2.77% difference between the official method and the pick both method, we see real accuracy issues, which can cost seed growers many dollars, and cause the buyer to purchase more seed than necessary (Table 2). The total viability means of the 10 samples were 86% official method (OM), 85% pick light method (PL), and 87% pick both method (PB) shown in table 4.

Table 5: Financial Impact on the Seed Industry between Three Purity Methods

	Official Method (OM)	Pick Light (PL)	Pick Both (PB)
Purity% Mean of 10 lots	69.73	73.14	72.50
Total Viability Mean of 10 lots	86	85	87
PLS=(Purity X Total Viable)/100	59.67	62.17	63.08
PLS on 20,000# lot	11,934	12,435	12,616
\$/PLS	8.50	8.50	8.50
Total \$ value of lot	\$101,439	\$105,697	\$107,236
\$ loss compared to OM		\$-4,258	\$-5,797

Table 5 shows that when comparing the official method (OM) to the pick light method (PL), the average decreased value of the 20,000 lb. (average size) seed lots is \$-4,258. When comparing the official method (OM) to the pick both method (PB), the average decreased value of the 10 seed lots is \$-5,797.

Conclusion:

Answers to the objectives stated for this study follow:

1. All 10 samples had florets with caryopses blown to the light portion when the official method (OM) was applied. Table 3 shows a range of 4 to 352 florets with caryopses blowing into the light portion.

2. Nine of ten samples had varying amounts of empty florets in the heavy portion of the official method (OM). Table 3 shows a range of 0 to 119 empty florets found in the heavy portion.
3. There was a wide range on the net effect of removing florets with caryopses from the light portion and empty florets from the heavy portion of the official method (OM). Table 3 shows the range was - 0.0139 to 0.1570 grams net difference.
4. Four of the ten samples tested were out of tolerance (Table 2) according the table 13A of the AOSA rules. Nine of the ten samples resulted in higher purity percentages by hand picking vs. the official method (Table 2).
5. Fine-tune the blowing method to be more accurate: The results of this study indicate the major area to “fine-tune” is hand-picking the light portion for florets with caryopses. There might be times where the heavy portion should also be hand-picked. The results suggest that something needs to be done to improve the blowing procedure of blue grama. A protocol involving hand-picking the light portion in all cases, and determining a way to selectively hand-pick the heavy portion when needed, should be explored.

Based on the above studies, fine-tuning the current blowing procedure as proposed will produce accurate, consistent and efficient results. It is the seed grower who is being hurt the most, since seed companies buy on the test results based on the AOSA rules. Many dollars have been lost in the blue grama seed industry, and saving a few dollars on a more efficient test with the official method today, is far out-weighted by the losses to the seed growers. The seed industry has been asking for a solution to this problem since we began using the blowing point procedure in 1981, and this study has reinforced their concern.

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