2011 Rules Change Proposal 1

Purpose of Proposal: To add a definition for dead seeds to Volume 1, of the AOSA rules, Principal and Procedures, Section 6 (Germination tests).

Present Rule:

6.2 Definitions

a. **Seed germination.** – In seed laboratory practice, germination is defined as the emergence and development from the seed embryo of those essential structures that, for the kind of seed in question, are indicative of the ability to produce a normal plant under favorable conditions. Refer to section 6.2 d and e.

b. **Normal seedling. –** Seedlings possessing the essential structures that are indicative of their ability to produce plants under favorable conditions.

c. **Abnormal seedlings.** – All seedlings that cannot be classified as normal seedlings.

d. Hard seeds. – Seeds that remain hard at the end of the prescribed test period because they have not absorbed water due to an impermeable seed coat. Seeds known and recognized to contain hard seed are indicated in the "Fresh and Dormant Seed" column of Table 6A. The percentage hard seed is to be reported in addition to the percentage germination.

Proposed Rule:

6.3 Definitions

a. Seed germination. – In seed laboratory practice, germination is defined as the emergence and development from the seed embryo of those essential structures that, for the kind of seed in question, are indicative of the ability to produce a normal plant under favorable conditions. Refer to section 6.2 d and e.

b. Normal seedling. – Seedlings possessing the essential structures that are indicative of their ability to produce plants under favorable conditions.

c. Abnormal seedlings. – All seedlings that cannot be classified as normal seedlings.

d. Hard seeds. – Seeds that remain hard at the end of the prescribed test period because they have not absorbed water due to an impermeable seed coat. Seeds known and recognized to contain hard seed are indicated in the "Fresh and Dormant Seed" column of Table 6A. The percentage hard seed is to be reported in addition to the percentage germination.

e. Dead seeds. -- Seeds that at the end of the test period are neither hard nor dormant nor have produced any part of a seedling.

Harmonization:
This addition of a dead seed definition will improve harmonization with existing rules of other organizations. The Canadian Food Inspection Agency Methods and Procedures for Testing Seeds (CFIA M&P), has the dead seed definition of "Seeds which at the end of the test period are neither hard nor dormant nor have produced any part of a seedling." The International Seed Testing Association Rules for Seed Testing (ISTA Rules) has the dead seed definition of "Seeds which at the end of the test period are neither hard nor fresh nor have produced any part of a seedling."

Supporting evidence:
This definition is important to properly characterize dead seeds in a germination test. In Volume 1 of the AOSA rules, Principles and Procedures, in Section 6.5 (Evaluation of Seedlings) it states that "During the progress of the germination test, seeds that are obviously dead and moldy and that may be a source of contamination of healthy seeds should be removed at each count and the number of such dead seeds should be recorded." In Volume 1of the AOSA rules, Principles and Procedures, in Section 6.7(Calculation of percentage germination), dead seeds are referred to seven times, and all of the calculation rules depend on counting dead seeds. In Volume 4 of the AOSA rules, Seedling Evaluation, the glossary defines dead seeds as "Seeds that at the end of the test period are neither hard nor dormant nor have produced any part of a seedling." It is therefore proposed to add the same definition to Volume 1 of the AOSA rules, Principles and Procedures to Section 6 (Germination tests).

Submitted by:
Brenda R. Watts, RST
Dairyland Seed Co.
504 W. Idaho Ave.
Homedale, Idaho 83628
Phone: 208 337-4693
Fax: 208 337-4168
Email: brwatts@dairylandseed.com

Date submitted: September 9, 2010

2011 Rules Change Proposal 2 Amended

Purpose of the Proposal: To eliminate paired germination testing and pre-chilling as a specific requirement on *Linum lewisii*.

Present Rule:

Kind of seed	Substrata	Temp. (°C)	First count (days)	Second count (days)	Specific requirements and notes	Fresh and dormant seed
Linum lewisii Lewis flax	TB	15; 10-20	10	28	Light. Paired tests. Prechill 28 days at 2-5°C	Ungerminated seeds: see sec. 6.2e and 6.9k

Proposed Rule:

Kind of seed	Substrata	Temp. (°C)	First count (days)	Second count (days)	Specific requirements and notes	Fresh and dormant seed
Linum lewisii Lewis flax	TB	15; 10-20	10	28	Light. ~~Paired tests. Prechill 28 days at 2-5°C~~	Ungerminated seeds: see sec. 6.2e and 6.9m

Harmonization: This species is not covered by the Federal Seed Act, the Canadian Methods and Procedures for Testing Seeds, or the International Seed Testing Association Rules for Seed Testing.

Supporting Evidence: (See appendix) Germination results were recorded and analyzed for 88 Lewis flax samples that have been tested over a 4-year period. The Lewis flax samples originated from different sources, differed in quality (germination percentage), and were tested at various periods after harvest (Table 1). All samples were germinated according to AOSA rules and guidelines with and without a prechilling treatment.

Overall mean percentage germination, combining all sample results, indicated that prechilling had no effect on germination ($p \leq 0.01\%$) and that percentage germination with and without prechilling did not significantly differ (Table 2). As a matter of fact, absence of pre-chilling resulted in a significantly higher number of cases (Chi-square test, $p \leq 0.01\%$) with improved germination (Table 3), although the improvement in germination was minimal. When samples were classified

according to length of period from harvest to testing, year of testing and quality, pre-chilling did not result in any significant improvement in germination for any of the examined categories (Figures 1, 2 and 3)

Pre-chilling *Linum lewisii* is not an efficient use of time or material and has neither a positive nor negative effect on germination results. Results will be reported 28 days sooner if eliminated and it would save money on resources and materials used for planting. If approved, the proposed rule will still provide an alternative germination procedure when dormancy is detected.

Submitted By:

Amanda Boehler (aboehler@agr.wa.gov)
Chris Norvell (Cnorvell@agr.wa.gov)
Linda Rainey (Lrainey@agr.wa.gov)
Victor Shaul (vshaul@agr.wa.gov)
Fawad Shah (fshah@agr.wa.gov)
Washington State Department of Agriculture
Seed Program
21 North 1st Avenue, Suite 203
Yakima, WA 98902
Phone:(509)249.6950
FAX:509.454.4395

Riad Baalbaki (rbaalbaki@cdfa.ca.gov)
Plant Pest Diagnostics Branch
California Department of Food and Agriculture
3294 Meadowview Road
Sacramento, CA 95832-1448
Phone: (916) 262-3292
Fax: (916) 262-1190

Date Submitted: July 16, 2010

2011 Rules Change Proposal 3

Purpose of Proposal: To add *Encelia* spp. to the Uniform Classification Table of the AOSA Rules.

Present Rule:

AOSA Rules for Testing Seeds Volume 3. Uniform Classification of Weed and Crop Seeds -- Uniform Classification Sorted by Scientific Name:

NOMEN #	SCIENTIFIC NAME	COMMON NAME	FAMILY	SPP. CLASS	CONTAMINATING CLASSIFICATION						
					A	F	H	R	S	T	V
447408	*Encelia californica* Nutt.	sunflower, California	Asteraceae	F, R, S	W	C	W	C	C	W	W
15137	*Encelia farinosa* A. Gray ex Torr.	brittlebush	Asteraceae	F, R, S	W	C	W	C	C	W	W
15138	*Encelia frutescens* (A. Gray) A. Gray	encelia, bush; brittlebush, button; brittlebush	Asteraceae	F, R, S	W	C	W	C	C	W	W
448135	*Encelia virginensis* A. Nelson	brittlebush, Virgin River	Asteraceae	F, R, S	W	C	W	C	C	W	W

Proposed Rule:

AOSA Rules for Testing Seeds Volume 3. Uniform Classification of Weed and Crop Seeds -- Uniform Classification Sorted by Scientific Name:

NOMEN #	SCIENTIFIC NAME	COMMON NAME	FAMILY	SPP. CLASS	CONTAMINATING CLASSIFICATION						
					A	F	H	R	S	T	V
447408	*Encelia californica* Nutt.	sunflower, California	Asteraceae	F, R, S	W	C	W	C	C	W	W
15137	*Encelia farinosa* A. Gray ex Torr.	brittlebush	Asteraceae	F, R, S	W	C	W	C	C	W	W
15138	*Encelia frutescens* (A. Gray) A. Gray	encelia, bush; brittlebush, button; brittlebush	Asteraceae	F, R, S	W	C	W	C	C	W	W
448135	*Encelia virginensis* A. Nelson	brittlebush, Virgin River	Asteraceae	F, R, S	W	C	W	C	C	W	W
	Encelia spp.	brittlebush	Asteraceae	F, R, S	W	C	W	C	C	W	W

Harmonization and Impact Statement: This proposal does not harmonize with ISTA, Canadian methods, or the Federal Seed Act. ISTA classifies all contaminating species as other seed; the Federal Seed Act leaves the classification of contaminants up to the individual states; and the Canadian Methods and Procedures do not list *Encelia* spp. as a crop.

Supporting Evidence: The genus *Encelia* consists of 13 species of small shrubs native to western North and South America. Currently the AOSA Rules Uniform Classification lists four species of *Encelia*, all of which are native to California and the southwestern United States. The seeds of these species are very difficult to distinguish from one another. There is no evidence of problems of invasiveness within this genus. It would be very helpful to classify the entire genus in the same way that the four species currently included have been classified because of the difficulty in seed identification of *Encelia* to the species level when found as a contaminant.

References:

Germplasm Resources Information Network (GRIN):
http://www.ars-grin.gov/cgi-bin/npgs/html/tax_search.pl

Hickman, James (ed.). 1993. The Jepson Manual: Higher Plants of California. University of California Press.

PLANTS Database of the UDSA Natural Resources Conservation Service:
http://plants.usda.gov/java/profile?symbol=ENCEL

Submitted By:

Sue Alvarez, R.S.T.
Ransom Seed Laboratory
P.O. Box 300
Carpinteria, California 93014-0300
Phone: 805-684-3427
Fax: 805-684-4157
Email: RansomSL@silcom.com

Date Submitted: September 28, 2010

2011 Rules Change Proposal 4

Purpose of Proposal: To clarify the "Specific requirements" in Table 6A of the AOSA Rules, Volume 1, for *Centaurea americana*, *Cucurbita* spp., and *Lagenaria* spp., regarding clipping the radicle end of seeds, and to remove the "Hard seeds" note for *Centaurea americana*.

Present Rule:

AOSA Rules for Testing Seeds Volume 1. Principles and Procedures:

Table 6A. Methods of testing for laboratory germination.

Kind of Seed	Substrata[a]	Temperature (°C)	First count (days)	Final count (days)	Specific requirements and notes	Fresh and dormant seed
Centaurea americana basketflower	B, T	20-30	5[b]	14[c]	Clip radicle end of seed for rapid response. Hard seeds: see sec. 6.2d and 6.9m(6)	
Cucurbita spp., etc. gourds: small-seeded large-seeded	TB, B, T TB, B, T	20-30 20-30	3[b] 4[b]	7[c] 10[c]	Some cultivars need radicle end of seed clipped for prompt response	
Lagenaria spp., etc. gourd: small-seeded large-seeded	TB, B, T TB, B, T	20-30 20-30	3[b] 4[b]	7[c] 10[c]	Some cultivars need radicle end of seed clipped for prompt response	
Sorghum 'Sorgrass'[d] sorgrass	B, T, S	20-35; 15-35	5	21		Prechill at 5°C for 5 days

[a] For coated seed, pleated paper (PP) may be used instead of the listed substrata. See sec. 6.8 l (1).
[b] Make preliminary counts only when necessary or advisable for efficiency. Many flower seedlings can be judged more accurately and critically if seedlings are left for final count, especially in test durations of 7-14 days.
[c] Final count may vary with certain types, cultivars, or strains within any flower seed kind. Remaining seeds at the end of the test should be critically evaluated for any viable seeds and recorded as dormant (see section 6.9 m).
[d] Rhizomatous derivatives of a johnsongrass x sorghum cross or a johnsongrass x sudangrass cross.

Proposed Rule:

AOSA Rules for Testing Seeds Volume 1. Principles and Procedures:

Table 6A. Methods of testing for laboratory germination.

Kind of Seed	Substrata[a]	Temperature (°C)	First count (days)	Final count (days)	Specific requirements and notes	Fresh and dormant seed
Centaurea americana basketflower	B, T	20-30	5[b]	14[c]	Clip radicle end of seed coat for rapid response[d]	
Cucurbita spp., etc. gourds: small-seeded large-seeded	TB, B, T TB, B, T	20-30 20-30	3[b] 4[b]	7[c] 10[c]	Some cultivars need radicle end of seed coat clipped for prompt response[d]	
Lagenaria spp., etc. gourds: small-seeded large-seeded	TB, B, T TB, B, T	20-30 20-30	3[b] 4[b]	7[c] 10[c]	Some cultivars need radicle end of seed coat clipped for prompt response[d]	
Sorghum 'Sorgrass'[e] sorgrass	B, T, S	20-35; 15-35	5	21		Prechill at 5°C for 5 days

[a] For coated seed, pleated paper (PP) may be used instead of the listed substrata. See sec. 6.8 l (1).

[b] Make preliminary counts only when necessary or advisable for efficiency. Many flower seedlings can be judged more accurately and critically if seedlings are left for final count, especially in test durations of 7-14 days.
[c] Final count may vary with certain types, cultivars, or strains within any flower seed kind. Remaining seeds at the end of the test should be critically evaluated for any viable seeds and recorded as dormant (see section 6.9 m).
[d] Avoid injury to the radicle or other embryo structures.
[e] Rhizomatous derivatives of a johnsongrass x sorghum cross or a johnsongrass x sudangrass cross.

Harmonization and Impact Statement:

This proposal will have no impact on harmonization since it serves as a rule clarification, not a rule change proposal. ISTA rules do not include a requirement for clipping the radicle end for any of the listed species, and neither the Federal Seed Act nor the Canadian Methods and Procedures include any of the above species.

This rule clarification will further standardize germination test procedures among laboratories. Current instructions may lead analysts to clip the actual tip of the embryonic radicle, resulting in induced seed death or seedling abnormalities, underestimating the germination potential of the tested sample.

Supporting Evidence:
The instructions to clip the radicle end have been in the rules a long time (at least since 1965) and are correct but misleading. What needs to be clipped is the seed coat at the radicle end, not the radicle itself. Some seeds have a physical plug made of hard material at the radicle end. This acts as a physical barrier to radicle emergence even though the seed can take up water.

Species recognized in the AOSA Rules as having “hard seed” are members of the Convolvulaceae. Fabaceae, Geraniaceae, and Malvaceae families. *Centaurea americana* is a member of the Asteraceae family, which is not recognized as having “hard seed,” so the note referring to *C. americana* hard seed should be removed. It is suspected that this species’ inability to germinate, remaining firm because of this plug, has been wrongly confused as a hard seed condition. Many species with physical barriers appear hard but if carefully inspected it is easy to tell that they have taken up water but

remained firm because of physical resistance to expansion (as opposed to hard seeds that do not take up water).

Submitted By:

Riad Baalbaki, Senior Seed Botanist
California Department of Food and Agriculture
Plant Pest Diagnostics Branch
3294 Meadowview Road
Sacramento, CA 95832-1448
Phone: (916) 262-3292
Fax: (916) 262-1190
Email: RBaalbaki@cdfa.ca.gov

Sue Alvarez, R.S.T.
Ransom Seed Laboratory
P.O. Box 300
Carpinteria, California 93014-0300
Phone: 805-684-3427
Fax: 805-684-4157
Email: RansomSL@silcom.com

Date Submitted: September 29, 2010

2011 Rules Change Proposal 6

Purpose of Proposal: To change the pure seed unit definition for *Achillea millefolium* (common yarrow) from PSU Number 27 to PSU Number 50.

Present Rule:

AOSA Rules for Testing Seeds Volume 1. Principles and Procedures:

Table 2A. Weights for working samples.

Pure Seed Unit #	Kind of seed	Minimum weight for purity analysis	Minimum weight for noxious-weed seed or bulk examination	Approximate number of seeds per gram	Approximate number of seeds per ounce
		Grams	Grams	Number	Number
27	*Achillea millefolium* L. common yarrow	0.4	4	6,325	179,314

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
27	Intact achene whether or not a seed is present. Piece of broken achene larger than one-half of the original size, unless no seed is present. Seed, with or without pericarp/seed coat. Piece of broken seed, with or without pericarp/seed coat, larger than one-half the original size.

Proposed Rule:

AOSA Rules for Testing Seeds Volume 1. Principles and Procedures:

Table 2A. Weights for working samples.

Pure Seed Unit #	Kind of seed	Minimum weight for purity analysis	Minimum weight for noxious-weed seed or bulk examination	Approximate number of seeds per gram	Approximate number of seeds per ounce
		Grams	Grams	Number	Number
50	*Achillea millefolium* L. common yarrow	0.4	4	6,325	179,314

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
50	Intact achene, with or without one or more of the following structures: beak, bristle, hairs, pappus, wing, or firmly attached floral remnants, provided a true seed with some degree of embryo development can be detected (either by slight pressure or by examination over light). Piece of broken achene larger than one-half of the original size, unless no seed is present. Seed, with or without pericarp/seed coat. Piece of broken seed, with or without pericarp/seed coat, larger than one-half the original size.

Harmonization and Impact Statement: This proposal does not harmonize with ISTA, Canadian methods, or the Federal Seed Act.

Supporting Evidence: In 2007, PSU 50 was added to the AOSA Rules to better describe pure seed units for some "native" species in the Asteraceae family. At that time only a few species were changed from PSU 27 or 28 to PSU 50, with the idea of adding additional appropriate species as they are identified or added to the Rules. *Achillea millefolium* is a member of the Asteraceae family native to much of western North America. The seed unit is an achene with no pappus. The fruit wall is transparent and the seed (embryo) can easily be seen with the aid of a diaphanoscope. This seed unit fits nicely under the definition given under PSU 50. See accompanying photograph (Figure 1).

References:

Bailey, LH (ed.) 1976. Hortus Third – A Concise Dictionary of Plants Cultivated in the United States and Canada. Macmillan Publishing Company.

Hickman, James (ed.). 1993. The Jepson Manual: Higher Plants of California. University of California Press.

Figure 1: *Achillea millefolium* with bottom light

Submitted By:

Michael Aberle, R.S.T
Sue Alvarez, R.S.T.

Ransom Seed Laboratory
P.O. Box 300
Carpinteria, California 93014-0300
Phone: 805-684-3427
Fax: 805-684-4157
Email: RansomSL@silcom.com

Date Submitted: September 29, 2010

2011 Rules Change Proposal 7

Purpose: To correct the improper assignment of the pure seed unit definition for *Calocedrus decurrens,* incense cedar. The assignment will change from PSU 5 to PSU 4.

Present rule:

Table 2.4 Weights for working samples.

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
5	*Calocedrus decurrens* (Torr.) Florin incense cedar	87	-	29	815

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
5	Seed, with at least a portion of the seed coat attached, without wing except for the part that encloses the seed. Piece of broken seed larger than one-half the original size with at least a portion of the seed coat attached. Special consideration: • Pieces of wing not enclosing the seed are removed and considered inert matter.

Proposed rule:

Table 2.4 Weights for working samples.

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
4	*Calocedrus decurrens* (Torr.) Florin incense cedar	87	-	29	815

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
4	Seed with at least a portion of the seed coat attached, with or without wing(s). Piece of broken seed larger than one-half the original size with at least a portion of the seed coat attached.

Harmonization and impact statement: This species is not covered by the Federal Seed Act or the Canadian Food Inspection Agency Methods, Procedures for Testing Seeds. The International Seed Testing Association Rules for Seed Testing includes this species and the proposed change will bring the AOSA and ISTA rules into harmony.

Supporting evidence: The seeds of *Calocedrus decurrens* have two persistent wings that are not easily removed without causing damage to the seed. This seed is sold commercially with and without the wing based on customer request (Vankus, personal communication).

The ISTA Rules PSD 49 defines the seed units as follows: Seed, with or without wing(s), provided a portion of the testa is attached. Piece of seed larger than one-half the original size, provided a portion of the testa is attached.

Submitted by: Victor Vankus, Tree and Shrub Subcommittee, USDA Forest Service National Seed Laboratory, (478) 751-3551, vvankus@fs.fed.us, and Deborah Meyer, Purity Subcommittee Chair, California Department of Food and Agriculture, (916) 262-1137, dmeyer@cdfa.ca.gov.

Date Submitted: October 15, 2010

Calocedrus decurrens, incense cedar seed. Seed at top with most of the wing tissue removed. Seed at bottom with two intact and persistent wings.

2011 Rules Change Proposal 8

Purpose: To reassign *Pinus echinatus (*shortleaf pine*), P. elliottii* (slash pine), *Pinus rigida* (pitch pine), and *P. taeda* (loblolly pine) from Pure Seed Unit 5 to PSU 2 because these species are marketed in the de-winged format.

Present rule:

Table 2.4 Weights for working samples.

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
5	*Pinus echinata* Mill. shortleaf pine	28	-	88	2,505
5	*Pinus elliottii* Engelm. slash pine	96	-	26	735
5	*Pinus rigida Mill.* pitch pine	20	-	135	3,880
5	*Pinus taeda L.* loblolly pine	67	-	38	1,065

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
5	Seed, with at least a portion of the seed coat attached, without wing except for the part that encloses the seed. Piece of broken seed larger than one-half the original size with at least a portion of the seed coat attached. Special consideration: • Pieces of wing not enclosing the seed are removed and considered inert matter.

Proposed Rule:

Table 2.4 Weights for working samples.

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
2	*Pinus echinata* Mill. shortleaf pine	28	-	88	2,505

2	*Pinus elliottii* Engelm. slash pine	96	-	26	735
2	*Pinus rigida Mill.* pitch pine	20	-	135	3,880
2	*Pinus taeda L.* loblolly pine	67	-	38	1,065

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
2	Seed with at least a portion of the seed coat attached. Broken seed larger than one-half the original size with at least a portion of the seed coat attached. Special considerations: • For Fabaceae: Cotyledons that are broken apart but held together by the seed coat shall be classified as pure seed. Cotyledons that have separated and are not held together by the seed coat are regarded as inert matter irrespective of whether or not the radicle-plumule axis and/or more than half of the seed coat may be attached. • Wing, when present, is removed and considered inert matter. • Pericarp (fruit wall), when present on seeds of *Desmodium tortuosum*, *Hedysarum boreale*, and *Purshia tridentata* is removed and considered inert matter. • Weevil-infested vetch (*Vicia* spp.) and pea (*Pisum sativum*) seeds, irrespective of the amount of insect damage, are to be considered pure seed, unless they are broken pieces one-half the original size or less. • Chalcid-damaged seeds in Fabaceae that are puffy, soft, or dry and crumbly are considered inert matter.

Harmonization and impact statement: These species are not covered by the Federal Seed Act or the Canadian Food Inspection Agency Methods and Procedures for Testing Seeds. The International Seed Testing Association Rules for Seed Testing does include these species, but the ISTA and AOSA rules differ in the definition of the pure seed unit and in how to report winged seed. The proposal, although it would correctly recognize that the pure seed should be without a wing, as in the ISTA Rules, will continue to differ because the occasional winged seed found in purity analysis will continue to be treated differently (refer to ISTA Rules sections shown below). Also, for *Pinus rigida* the proposed seed unit definition will differ from the ISTA definition because under ISTA it is permissible to have the portion of the wing immediately surrounding the seed remain as part of the pure seed unit.

Supporting evidence: The seeds of these four species are marketed in the de-winged format and after conditioning are rarely found with the wing remaining attached. Gymnosperms have only one integument, which becomes differentiated into three zones or layers: an inner fleshy layer, a middle sclerenchymatous layer, and an outer fleshy layer (Foster and Gifford, 1974). In *Pinus* the middle sclerenchymatous layer

becomes the hard ‘shell’ of the seed coat (testa) (Raven, et al., 1999). The wing is formed by separation of a portion of the upper surface of the cone scale adjacent to the ovule; therefore, the wing is not morphologically part of the seed (Sporne, 1965; Judd et al. 1999). For the four species considered here, the wing is easily detached from the seed during conditioning. If seeds with wings are found during a purity analysis, the proposed PSU definition will require the wing be removed from the seed.

Pinus echinata, winged seed (bottom), de-winged seed classified as pure seed (middle) and wing without seed classified as inert matter (top).

Given the references cited above, the definitions in the ISTA Rules are troubling in that the structure described as the ‘integument’ appears to be part of the ovulate cone scale, as is the wing; however, it appears the intent is the same as in AOSA PSU 2 (i.e., the wing, including the portion surrounding the seed, is classified as inert matter).

ISTA wording:

PSD 47
Seed, without wing or integument, provided a portion of the testa is attached.
Piece of seed larger than one-half the original size, without wing or integument, provided a portion of the testa is attached.
N.B. ‘Integument’ refers to the tissue attaching the wing to the seed. In Pinaceae with PSD 47, the integument is not intimately associated with the seed and is usually removed in processing, thus removing the wing. However, if an integument (with or without wing) is still attached to any seed during the purity analysis, such seed will be regarded as ‘winged seed and must be left intact; neither the integument nor wing should be deliberately removed. Winged seed (i.e., seed with an attached integument with or without a wing of any size) must be weighed and reported as a separate percentage from ‘pure seed’ according to paragraphs 3.5.2.9 and 3.7. After weighing, the winged seed and pure seed fractions should be recombined and used in representative proportions for counting out the germination replicates.

PSD 51
Seed, without wing, with (but occasionally without) integument, provided a part of the testa is attached.
Piece of seed larger than one-half the original size, without wing, with (but occasionally without) integument, provided a portion of the testa is attached.
N.B. ‘Integument refers to the tissue attaching the wing to the seed. In Pinaceae with PSD 51, the integument is fused to or intimately associated with the seed, is rarely removed in processing, and is impossible to consistently remove without causing damage. Hence, seed with fused or intimately associated integument

attached is considered to be 'pure seed'. Winged seed (i.e. seed with an integument plus wing still attached) must be weighed and reported as a separate percentage from 'pure seed' according to paragraphs 2.5.2.9 and 3.7. After weighing, the winged seed and pure seed fractions should be recombined and used in representative proportions for counting out the germination replicates.

3.5.2.9 Winged seed
For seed with PSD 47, winged seeds are those which retain an integument, either with or without wing or a portion thereof. For seeds with PSD 51, winged seeds are those which retain the wing or a portion thereof. Whenever present, such appendages must be left attached to the seed and the content of 'winged' seed reported according to 3.7.

3.7 Reporting results
The results of the purity test must be reported in the spaces provided as follows:….
The percentage of winged seed (as defined in Pure Seed Definitions 47 and 51), if winged seeds are found.

References

Foster, A. S. and E. M. Gifford. 1974. Comparative morphology of vascular plants. 2nd ed. W. H. Freeman and Company, San Francisco, CA. 751 pp.

Judd, W., C. S. Campbell, E. A. Kellogg, P. F. Stevens. 1999. Plant systematics: A phylogenetic approach. Sinauer Associates, Inc., Sunderland, MA. 464 pp.

Raven, P. H., R. F. Evert, S. E. Eichhorn. Biology of plants. 6th ed. W. H. Freeman and Company/Worth Publishers. 944 pp.

Sporne, K. R. 1965. The morphology of Gymnopserms. Hutchinson University Library, London.

Submitted by: Victor Vankus, Tree and Shrub Subcommittee, USDA Forest Service National Seed Laboratory, (478) 751-3551, vvankus@fs.fed.us, and Deborah Meyer, Purity Subcommittee Chair, California Department of Food and Agriculture, (916) 262-1137, dmeyer@cdfa.ca.gov.

Date Submitted: October 15, 2010

2011 Rules Change Proposal 9

Purpose: To correct the improper assignment of the pure seed unit for *Thuja occidentalis*, northern white cedar, and *T. plicata*, western red cedar. The assignment will change from PSU 2 to PSU 4.

Present rule:

Table 2.4 Weights for working samples.

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
2	*Thuja occidentalis* L. northern white cedar, eastern arborvitae	3	-	765	21,600
2	*Thuja plicata* Donn ex D. Don western red cedar, giant arborvitae	3	-	915	25,900

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
2	Seed with at least a portion of the seed coat attached. Broken seed larger than one-half the original size with at least a portion of the seed coat attached. Special considerations: * For Fabaceae: Cotyledons that are broken apart but held together by the seed coat shall be classified as pure seed. Cotyledons that have separated and are not held together by the seed coat are regarded as inert matter irrespective of whether or not the radicle-plumule axis and/or more than half of the seed coat may be attached. * Wing, when present, is removed and considered inert matter. * Pericarp (fruit wall), when present on seeds of *Desmodium tortuosum*, *Hedysarum boreale*, and *Purshia tridentata* is removed and considered inert matter. * Weevil-infested vetch (*Vicia* spp.) and pea (*Pisum sativum*) seeds, irrespective of the amount of insect damage, are to be considered pure seed, unless they are broken pieces one-half the original size or less. * Chalcid-damaged seeds in Fabaceae that are puffy, soft, or dry and crumbly are considered inert matter.

Proposed Rule:

Table 2.4 Weights for working samples.

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
4	*Thuja occidentalis* L. northern white cedar, eastern arborvitae	3	-	765	21,600
4	*Thuja plicata* Donn ex D. Don western red cedar, giant arborvitae	3	-	915	25,900

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
4	Seed with at least a portion of the seed coat attached, with or without wing(s). Piece of broken seed larger than one-half the original size with at least a portion of the seed coat attached.

Harmonization and impact statement: This species is not covered by the Federal Seed Act or the Canadian Food Inspection Agency Methods and Procedures for Testing Seeds. The International Seed Testing Association Rules for Seed Testing include these two species and the proposed definition will bring the AOSA and ISTA rules in harmony.

Supporting evidence: The seeds of these two species have persistent wings that are not easily removed without causing damage to the seed. The ISTA Rules PSD 49 defines the seed units as follows: Seed, with or without wing(s), provided a portion of the testa is attached. Piece of seed larger than one-half the original size, provided a portion of the testa is attached.

Submitted by: Victor Vankus, Tree and Shrub Subcommittee, USDA Forest Service National Seed Laboratory, (478) 751-3551, vvankus@fs.fed.us, and Deborah Meyer, Purity Subcommittee Chair, California Department of Food and Agriculture, (916) 262-1137, dmeyer@cdfa.ca.gov.

Date Submitted: October 15, 2010

Thuja plicata, western red cedar, giant arborvitae, seed with persistent lateral wings.

2011 Rules Change Proposal 10

Purpose: To assign all forms (i.e., 'in burs' and 'out of burs') of *Medicago polymorpha* (California burclover) and *M. arabica* (spotted burclover) to Pure Seed Unit 9 and to clarify the PSU 9 definition in cases where seed lots are marketed as hulled seed as opposed to seed lots marketed as pods or burs. The genera and species currently assigned to PSU 9 include: *Arachis, Kummerowia, Lespedeza, Medicago lupulina, M. arabica* ('in burs'), *M. polymorpha* ('in burs'), *Melilotus, Onobrychis, Trifolium alexandrinum, T. campestre, T. dubium, T. fragiferum, T. glomeratum, T. hirtum, T. incarnatum, T. lappaceum, T. pratense, T. resupinatum, T. subterraneum.*

Present rule:

Table 2.4 Weights for working samples.

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
	Medicago arabica (L.) Huds.				
9	spotted burclover: in bur	50	500	50	1,390
2	out of bur	5	50	550	15,620
	Medicago polymorpha L.				
9	California burclover: in bur	50	500		
2	out of bur	7	70	375	10,650

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
2	Seed with at least a portion of the seed coat attached. Broken seed larger than one-half the original size with at least a portion of the seed coat attached. Special considerations: • For Fabaceae: Cotyledons that are broken apart but held together by the seed coat shall be classified as pure seed. Cotyledons that have separated and are not held together by the seed coat are regarded as inert matter irrespective of whether or not the radicle-plumule axis and/or more than half of the seed coat may be attached. • Wing, when present, is removed and considered inert matter. • Pericarp (fruit wall), when present on seeds of *Desmodium tortuosum*, *Hedysarum boreale*, and *Purshia tridentata* is removed and considered inert matter. • Weevil-infested vetch (*Vicia* spp.) and pea (*Pisum sativum*) seeds, irrespective of the amount of insect damage, are to be considered pure seed, unless they are broken pieces one-half the original size or less. • Chalcid-damaged seeds in Fabaceae that are puffy, soft, or dry and crumbly are considered inert matter.

9	Intact pod, with or without calyx or bracts, whether or not a seed is present. Piece of broken pod larger than one-half of the original size, unless no seed is present. Seed, with at least a portion of seed coat attached, with or without aril. Piece of broken seed larger than one-half the original size, with at least a portion of seed coat attached, with or without aril. Special consideration: For Fabaceae: • Cotyledons that are broken apart but held together by the seed coat shall be classified as pure seed. • Cotyledons that have separated and are not held together by the seed coat are regarded as inert matter irrespective of whether or not the radicle-plumule axis and/or more than half of the seed coat may be attached. • Chalcid-damaged seeds in Fabaceae that are puffy, soft, or dry and crumbly are considered inert matter.

Proposed rule:

Table 2.4 Weights for working samples.

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
9	*Medicago arabica* (L.) Huds. spotted burclover: in bur out of bur	 50 5	 500 50	 50 550	 1,390 15,620
9	*Medicago polymorpha* L. California burclover: in bur out of bur	 50 7	 500 70	 375	 10,650

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
9	Intact pod, with or without calyx or bracts, whether or not a seed is present. Piece of broken pod larger than one-half of the original size, unless no seed is present. Seed, with at least a portion of seed coat attached, with or without aril. Piece of broken seed larger than one-half the original size, with at least a portion of seed coat attached, with or without aril. Special consideration: For seed lots marketed as hulled seed: occasional intact or broken fruits are to be opened and the seeds with at least a portion of the seed coat intact are to be classified as pure seed and the remaining fruit structure classified as inert matter.

	For seed lots marketed as fruits (burs or pods), the fruits are to be classified as pure seed if they fit the criteria stated above. For Fabaceae: • Cotyledons that are broken apart but held together by the seed coat shall be classified as pure seed. • Cotyledons that have separated and are not held together by the seed coat are regarded as inert matter irrespective of whether or not the radicle-plumule axis and/or more than half of the seed coat may be attached. • Chalcid-damaged seeds in Fabaceae that are puffy, soft, or dry and crumbly are considered inert matter.

Harmonization and impact statement: The Federal Seed Act (FSA) section 201.47a(d) contains a statement regarding the removal of incidental legume pods when they are found in the working sample taken from a seed lot that is marketed as hulled seed. The proposed wording addition would harmonize the AOSA and FSA pure seed definitions for the kinds under PSU 9.

The Canadian Food Inspection Agency Methods, Procedures for Testing Seeds section 3.2.1 mentions one-and two-seeded pods of small legumes as structures also accepted under the broad definition of seed. No mention is made as to what should be done in cases where incidental pods are found in working samples drawn from seed lots marketed as hulled seed.

By pure seed definition, the International Seed Testing Association Rules for Seed Testing allows for hulled seeds only for *Medicago*, *Trifolium*, and *Arachis*, while allowing seeds and pods for *Melilotus*, *Lespedeza*, and *Onobrychis*. Further discussion can be found under supporting evidence.

Supporting evidence:
In the AOSA rules there is an interesting situation that needs to be resolved regarding legumes that are marketed in more than one seed unit format. Under PSU2 we have a list of species which are usually marketed as hulled seeds ('out of bur'). Accordingly, under PSU2 when an occasional fruit is found during the purity analysis it is opened and the seed is classified as pure seed and the remaining fruit structures are classified as inert matter. Under PSU9 we have a list of species that can be marketed as hulled seed or as fruits. If marketed as hulled seed, the occasional fruit that may appear in a purity analysis is left intact and classified as pure seed. If marketed as fruits (burs, pods, etc.) the fruits are left intact and classified as pure seed along with any occasional hulled seed.

Last year there was considerable discussion about the proper seed unit for *Arachis* (peanut). The FSA considers the intact pod a pure seed unit; therefore, AOSA also recognizes the intact pod as a pure seed unit. The issue with peanut is no different than any other legume crop assigned to PSU9. Take for example black medic, a single-seeded pod. If a seed lot of this species is marketed as a pod we do not open the pods

to look at the seeds because we assume a seed (with seed coat intact) is present if the pod is intact and it appears to be filled. The same would be true of peanut if the pods are intact and are being marketed as such; the pod is considered the pure seed unit (as long as the pod is intact the seeds are assumed to be intact).

Questions arise for legumes that are marketed in both the pod format and the hulled seed format. The main question is what to do with legume species that are marketed as hulled seeds if an occasional pod is found in the purity analysis. If the species is assigned to PSU2 the pod is always removed and classified as inert matter. If the species is assigned to PSU9 the pod is not removed and the whole fruit is classified as pure seed even if the majority of the seed lot consists of hulled seeds. The exceptions being *M. polymorpha* and *M. arabica*, but these two species are currently placed under PSU2 only if the seed lot is marketed as hulled seed. In Table 2A there are separated working weights for seeds 'in burs' versus 'out of burs' for these two species. If seed lots of these two species are marketed as burs (the legume pods is coiled and spiny) then they are assigned to PSU9.

To resolve the issue for peanut and other species marketed in different formats, one possible solution to this complex issue is to drop *M. polymorpha* and *M. arabica* from PSU2 (they would only appear under PSU9) and to add the following statement to PSU9:

> For seed lots marketed as hulled seed: occasional intact or broken fruits are to be opened and the seeds with at least a portion of the seed coat intact are to be classified as pure seed and the remaining fruit structure classified as inert matter. For seed lots marketed as fruits (burs or pods), the fruits are to be classified as pure seed if they fit the criteria stated above.

By adding this statement the AOSA Rules would be more closely harmonized with the FSA. In the FSA, section 201.47a Seed units, subsection (d) states: One- and two-seeded pods of small-seeded legumes (Leguminosae), bur of burclovers *(Medicago arabica*, *M. polymorpha*), and pods of peanuts (*Arachis hypogaea*). (This does not preclude the shelling of small-seeded legumes for purposes of identification.) Pods of legumes normally containing more than two seeds, when occurring incidentally in the working sample, should be hulled if the kind is hulled when marketed;

In the ISTA Rules, *Medicago*, *Arachis*, and *Trifolium* are assigned to PSD 11. Interestingly, ISTA provides separate working weights for *Medicago arabica* burs versus seeds; however, to the contrary the seed unit description does not include fruits. The description is as follows:

> Seed, provided a portion of the testa is attached.
> Piece of seed larger than one-half the original size, provided a portion of the test is attached.
> Seeds and pieces of seeds entirely without testa are regarded as inert matter.
> For Fabaceae (Leguminosae): separated cotyledons are regarded as inert matter irrespective of whether or not the radicle-plumule axis and/or more than half of the testa may be attached.

It should be mentioned that the ISTA Purity Committee is discussing whether the pure seed unit for peanut should also include seeds in pods since seed lots are marketed that way in some countries.

The ISTA Rules assign *Melilotus* and *Onobrychis* to PSD 21. Separate working weights are provided for fruits versus seeds of *Onobrychis*. In this instance the assigned PSD does include fruits. The description for PSD 21 is as follows:

> Pod, with or without calyx, with seed(s).
> Seed, provided a portion of the testa is attached.
> Piece of seed larger than one-half the original size, provided a portion of the testa is attached.
> Seeds and pieces of seed without testa are regarded as inert matter. Separated cotyledons are regarded as inert matter irrespective of whether or not the radicle-plumule axis and/or more than half of the testa may be attached.

The ISTA Rules assign *Lespedeza* (*Kummerowia*) to PSD 22. The description for PSD 22 is as follows:

> Pod, with or without calyx, with seed(s).
> Seed, provided a portion of the testa is attached.
> Piece of seed larger than one-half the original size, provided a portion of the testa is attached.
> For Fabaceae (Leguminosae): Seeds and pieces of seed without testa are regarded as inert matter. Separated cotyledons are regarded as inert matter irrespective of whether or not the radicle-plumule axis and/or more than half of the testa may be attached.

In conclusion, if the proposed statement is added to PSU9, then all options for the marketing of the legume species assigned to PSU9 are covered and should meet the standard in the seed industry today. Also, by adding the proposed statement to PSU9 the AOSA and FSA Rules would be more closely aligned.

Submitted by: Deborah Meyer, Purity Subcommittee Chair, California Department of Food and Agriculture, (916) 262-1137, dmeyer@cdfa.ca.gov.

Date Submitted: October 15, 2010; revised January 20, 2011

2011 Rules Change Proposal 11 Amended

Purpose: To modify the Pure Seed Unit 11.

Present and proposed rule:

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
11	Intact schizocarp or mericarp, with or without carpophore and/or pedicel, whether or not a seed is present. Piece of broken schizocarp or mericarp larger than one-half of the original size, unless no seed is present. Seed or piece of broken seed, with or without seed coat, larger than one-half the original size.

APPENDIX 4: GLOSSARY OF TERMS FOR PURE SEED UNITS

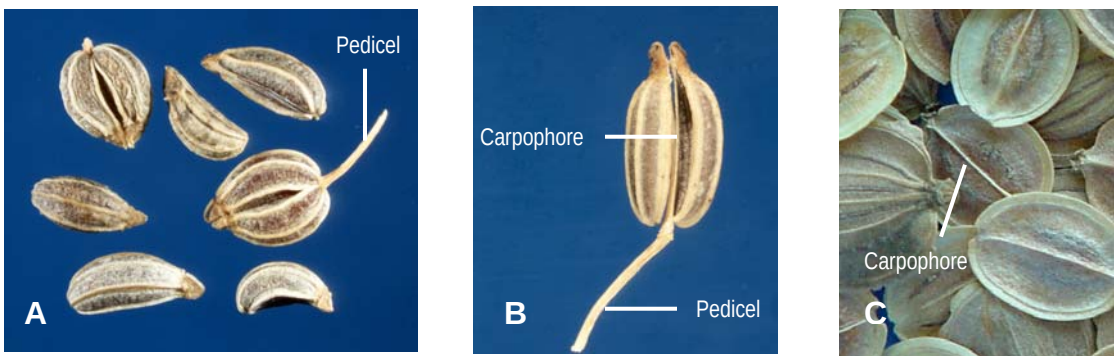
Carpophore. A slender extension of the receptacle between the two carpels of the fruits in some species of the Apiaceae or carrot family.

Harmonization and impact statement: The proposed addition to the definition for Pure Seed Unit 11 will harmonize with the International Seed Testing Association Rules for Seed Testing for members of the Apiaceae or carrot family. The Federal Seed Act and the Canadian Food Inspection Agency Methods, Procedures for Testing Seeds do not specifically mention carpophores or pedicels attached to the fruit in Apiaceae .

Supporting evidence: For kinds such as celery, fennel, parsley, parsnip, and others, a portion of the carpophore (a stem-like structure to which the two mericarps are attached in some species of the carrot family) and the pedicel may remain attached to the fruit. ISTA has recognized the tedious job involved in removing these structures during the purity analysis and has therefore included these naturally occurring structures as part of the recognized planting unit for members of the carrot family. The proposed addition to PSU 11 would harmonize the AOSA and ISTA pure seed unit definitions with respect to the attached stems for the carrot family. It should be noted that AOSA and ISTA Rules have always differed and will continue to differ with respect to whether a seed must be present in the intact fruit. In this matter AOSA PSU 11 remains in agreement with the pure seed definition in the Federal Seed Act.

Submitted by: Deborah Meyer, Purity Subcommittee Chair, California Department of Food and Agriculture, (916) 262-1137, dmeyer@cdfa.ca.gov.

Date Submitted: October 15, 2010; revised January 20, 2011



A – Parsley, B – Fennel, C – Parsnip fruits with attached pedicels and carpophores.

2011 Rules Change Proposal 12

Purpose: To modify Pure Seed Unit 16 to include a reference to the glossary for *rame internode* to be consistent with terminology used for other PSU definitions involving grasses that are members of the Andropogoneae.

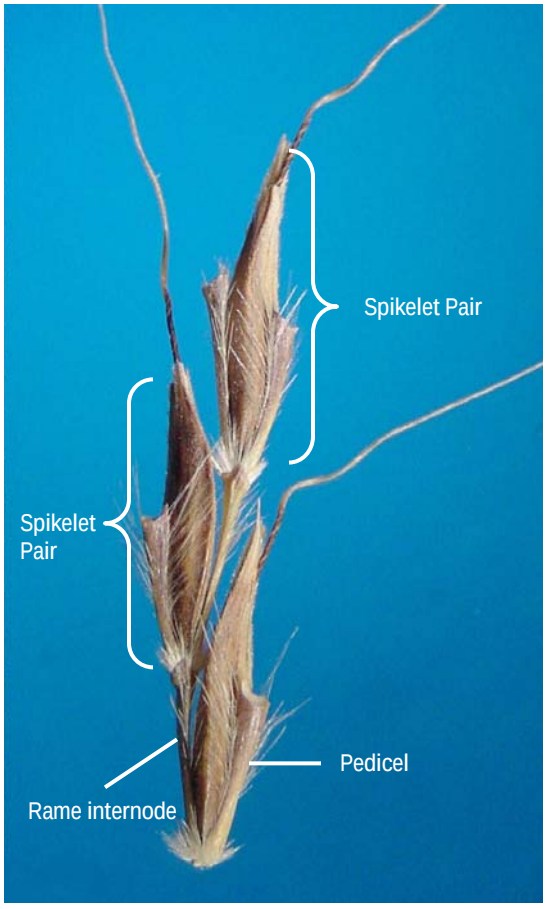
Present and proposed rule:

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
16	Spikelet group that disarticulates as a unit with attached rachis and internode, or spikelet with or without attached rachis segment (also see rame internode in glossary), pedicel and sterile spikelet, with or without awn(s), provided a caryopsis with some degree of endosperm development can be detected (either by slight pressure or by examination over light). Caryopsis or piece of broken caryopsis larger than one-half of the original size.

Harmonization and impact statement: The term rame internode corresponds with the terms *rachis* and *internodes* used in the Federal Seed Act (Sec. 201.4a(4)(ii)) to describe these structures. The International Seed Testing Association Rules for Seed Testing uses the term *rachis segments*. There is no specific mention of members of this group of grasses in the Canadian Food Inspection Agency Methods, Procedures for Testing Seeds.

Supporting evidence: The terms *rame* and *rame internode* are utilized in the treatment of members of the Andropogoneae provided by Barkworth, et al. (2003). To avoid confusion with recent publications these terms were added to the AOSA glossary in 2010.



Reference

Barkworth, M. E., K. M. Capels, S. Long, and M. B. Piep. 2003. Flora of North America, Volume 25, Magnoliophyta: Commelinidae (in part): Poaceae, part 2. Oxford University Press. 783 pp.

Submitted by: Deborah Meyer, Purity Subcommittee Chair, California Department of Food and Agriculture, (916) 262-1137, dmeyer@cdfa.ca.gov.

Date Submitted: October 15, 2010

2011 Rules Change Proposal 13

Purpose: To modify the definition for Pure Seed Unit 26 to only consider the size of seed-bearing portion of the fruit (samara) when evaluating the size of broken fruits in determining pure seed versus inert matter.

Present and proposed rule:

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
26	Intact samara ~~with or without wing(s),~~ with or without attached styles, whether or not a seed is present. Piece of broken samara larger than one-half of the original size (exclusive of the wing), unless no seed is present. Seed with or without pericarp/seed coat. Piece of broken seed, with or without pericarp/seed coat, larger than one-half the original size. Special consideration: • When determining the size of a piece of broken samara, the original size is based only on the size of the seed bearing portion of the samara. Any wing fragment without attached seed bearing portion is classified as inert matter.

Harmonization and impact statement: The genera of concern (*Acer, Ailanthus, Betula, Casuarina, Fraxinus, Liriodendron*, and *Ulmus*) are not covered by the Federal Seed Act or the Canadian Food Inspection Agency Methods, Procedures for Testing Seeds. The International Seed Testing Association Rules for Seed Testing includes these genera and the proposed change should not change the status of harmonization among these two sets of rules.

Supporting evidence: An intact samara includes the seed bearing portion of the fruit as well as the entire wing, as in *Acer, Ailanthus, Casuarina, Fraxinus, Liriodendron*, and *Ulmus*, or both entire wings, as in *Betula*. The statement 'intact samara with or without wing(s)' is contradictory because an intact samara cannot be without a wing, or even have a damaged wing. By removing the phrase 'with or without wing(s)' from the definition we can correct the error.

The second sentence of the definition, which describes damaged samaras, includes cases of damaged wings or the complete lack of a wing. A problem arises when a broken samara in which the seed bearing portion is intact or only slightly damage is less than one-half the size of the intact samara. It is likely such structures will be classified as inert matter even though they have the potential to produce a perfectly normal seedling. In the proposal, by excluding the wing, only the seed bearing portion of the samara is considered when evaluating the size of the remaining portion of the fruit.

ISTA has avoided the first problem by not using the term 'intact' to describe the seed unit; however, the second issue with regard to pieces of samaras (i.e., broken samaras) larger than on-half the original size may cause problems. It is not entirely clear from the ISTA definitions whether the wing(s) is considered when evaluating the size of the broken fruit, but based on the opening statement of each definition it is possible that the wing is discounted when making this determination.

The ISTA Rules PSD 52 defines the seed units as follows:
Samara (winged fruit), with or without wing(s).
Piece of samara larger than one-half the original size.
Seed with the pericarp/testa partially or entirely removed.
Piece of seed larger than one-half the original size, with the pericarp/testa partially or entirely removed.

The ISTA Rules PSD 53 defines the seed units as follows:
Samara (winged fruit), with or without wing(s), with or without attached styles.
Piece of samara larger than one-half the original size.
Seed, with the pericarp/testa partially or entirely removed.
Piece of seed larger than one-half the original size, with the pericarp/test partially or entirely removed.

Left to right: *Liriodendron, Ailanthus, Fraxinus*, and *Acer* samaras. Seed-bearing portion of each samara circled in red. These areas clearly constitute less than one-half the overall size of each intact samara. In cases where the wing is removed via conditioning or by other means, the seed-bearing portion could be classified as inert matter under the current PSU definition. By excluding the wing from consideration (as proposed), this seed bearing portion would then be classified as pure seed.

Submitted by: Victor Vankus, Tree and Shrub Subcommittee, USDA Forest Service National Seed Laboratory, (478) 751-3551, vvankus@fs.fed.us, and Deborah Meyer, Purity Subcommittee Chair, California Department of Food and Agriculture, (916) 262-1137, dmeyer@cdfa.ca.gov.

Date Submitted: October 15, 2010; revised January 20, 2011

2011 Rules Change Proposal 14

Purpose: To modify the definition for Pure Seed Unit 38 and add botanical terms to Appendix 4.

Present and proposed rule:

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
38	Intact utricle with or without perianth, enclosed or not enclosed by fruiting bracts (bracteoles), whether or not a seed is present. Piece of broken utricle larger than one-half the original size, unless no seed is present. Seed with or without seed coat. Piece of broken seed, with or without seed coat, larger than one-half the original size. Special consideration: • For Bassia prostrata, seed units that are retained on a 1mm opening square-hole sieve, when shaken for 30 seconds shall be considered pure seed units. Seed units that pass through the 1-mm sieve shall be classified as inert matter.

APPENDIX 4: GLOSSARY OF TERMS FOR PURE SEED UNITS

Bracteole – a small bract subtending a flower or fruit.

Fruiting bract – A small or rudimentary leaf or leaf-like structure near the base of a fruit that may or may not enclose the fruit.

Harmonization and impact statement: The species assigned to PSU 38 include *Atriplex canescens* (fourwing saltbush), *Bassia prostrata* (forage kochia), *B. scoparia*, (summer cypress), *Gomphrena globosa* (globe amaranth), and *Krascheninnikovia lanata* (winterfat). Of these species only fourwing saltbush and forage kochia are included in the Federal Seed Act. Under the FSA pure seed unit for fourwing saltbush comes under the general category for dry indehiscent one-seeded fruits, but there is no mention of surrounding structures. Forage kochia is treated the same way as under the AOSA Rules. The Canadian Food Inspection Agency Methods, Procedures for Testing Seeds does not include any of these species. The International Seed Testing Association Rules for Seed Testing includes globe amaranth and a different species of *Atriplex*. In the ISTA Rules globe amaranth is assigned to PSD 2; defined as follows: achene or cluster, with or without perianth or pedicel, unless it is obvious that no seed is present; piece

of achene or cluster larger than one-half the original size, unless it is obvious that no seed is present…..(*Gomphrena*: Achene with or without hairy perianth, unless it is obvious that no seed is present.). It is not clear what the term 'cluster' means.

Supporting evidence: For some members of the Chenopodiaceae and Amaranthaceae the fruit is surrounded by bracts (also referred to as fruiting bracts or bracteoles in the literature) and other persistent floral parts that fall together at maturity. The current AOSA PSU 38 definition does not include the fruiting bract; therefore, it must be removed. In winterfat that would mean removing the hairy bract, in fourwing saltbush that would mean removing (with great difficulty) the fruiting bracteoles, and in globe amaranth it would mean removing the colorful bracts subtending the persistent perianth. Since it is not practical to remove these structures during the purity analysis, the proposal is to allow these structures to remain as part of the pure seed unit. This is generally the way these kinds of seed are marketed (i.e., bracts intact). For more information on the morphological features of fourwing salt bush, winterfat, globe amaranth, and forage kochia, please see the attached excerpt (appendix) from the proposed AOSA Purity Testing Handbook. Please keep in mind that these pages are written with the assumption the proposed wording change for PSU 38 will be adopted.

Submitted by: Deborah Meyer, Purity Subcommittee Chair, California Department of Food and Agriculture, (916) 262-1137, dmeyer@cdfa.ca.gov.

Date Submitted: October 15, 2010; revised January 20, 2011

Appendix 1 to Proposal 14:

Pure Seed Unit 38

The genera assigned to PSU 38 belong to the goosefoot family (Chenopodiaceae) and amaranth family (Amaranthaceae) in which the fruit is a single-seeded dry indehiscent sac-like structure called a utricle. The utricle pericarp is usually thin and membranous. This structure is commonly enclosed by a persistent perianth or fruiting bracts (bracteoles), or both. The embryo is placed in the peripheral position and surrounds a firm, flinty, white nutritive tissue (perisperm) (Martin 1946).

Female flowers in *Atriplex canescens* (four-wing saltbush) lack petals and sepals. Instead, the one-seeded utricle is surrounded by two bracteoles (Figure 4.38.1). In most species the bracteoles enlarge in size as the fruit matures and become sealed around the fruit, but not fused to it (Meyer, 2008). The bracteoles are part of the seed unit and are not removed during the purity analysis. Filled and unfilled (lacking a fruit containing a seed) seed units are difficult to

Definition for PSU 38

Intact utricle with or without perianth, enclosed or not by fruiting bracts (bracteoles), whether or not a seed is present.

Piece of broken utricle larger than one-half the original size, unless no seed is present.

Seed with or without seed coat.

Piece of broken seed, with or without seed coat, larger than one-half the original size.

Special consideration:

- For *Bassia prostrata*, seed units that are retained on a 1mm opening square-hole sieve, when shaken for 30 seconds shall be considered pure seed units. Seed units that pass through the 1mm sieve shall be classified as inert matter.

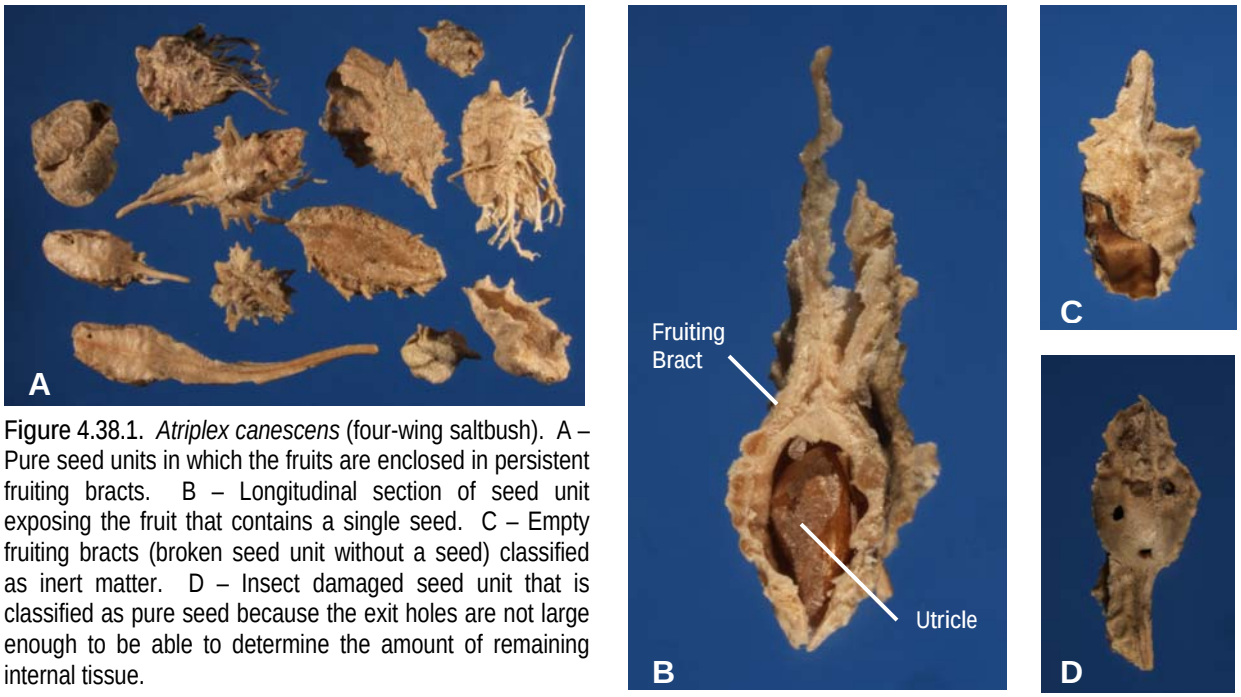


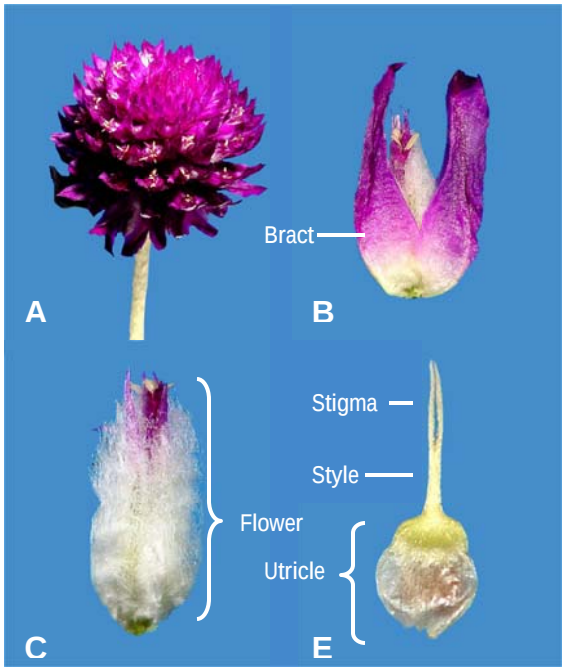
Figure 4.38.1. *Atriplex canescens* (four-wing saltbush). A – Pure seed units in which the fruits are enclosed in persistent fruiting bracts. B – Longitudinal section of seed unit exposing the fruit that contains a single seed. C – Empty fruiting bracts (broken seed unit without a seed) classified as inert matter. D – Insect damaged seed unit that is classified as pure seed because the exit holes are not large enough to be able to determine the amount of remaining internal tissue.

distinguish; therefore, intact unfilled seed units are classified as pure seed. Variation in seed unit size is not an indication of fruit fill (Meyer, 2008), which means some large seed units may not contain a seed, while some small seed units may be filled.

In *Krascheninnikovia lanata* (winterfat) the female flowers have two stigmas emerging from between two fruiting bracts (Booth, 2008). The fruiting bracts are densely covered with light tan hairs, except for the horned tips, and the utricle is also pubescent (Figure 4.38.2). Due to the super chaffy nature of these seed units it is difficult to determine whether the units are filled with a mature fruit containing a seed; therefore, intact utricles surrounded by fruiting bracts are classified as pure seed whether or not a seed is present. Preparing a proper working sample in the laboratory is difficult and the hand halving method is strongly recommended.

Figure 4.38.2. Winterfat (*Krascheninnikovia*) A – Seed units consisting of a single seeded utricle enclosed within two horn-tipped, densely pubescent fruiting bracts that may be further subtended by additional bracts. B – Utricle containing a single seed.

In *Gomphrena globosa* (globe amaranth) the inflorescence is composed of flowers surrounded by a pairs of colorful bracts (Figures 4.38.3 A and B). Within the colorful bracts, the five tepals of the perianth are covered by long silky hairs. The androecium is composed of five stamens, whose filaments form a membranous tube around the fragile utricle. The seed unit includes the perianth and subtending bracts; therefore, they are not removed during the purity analysis.



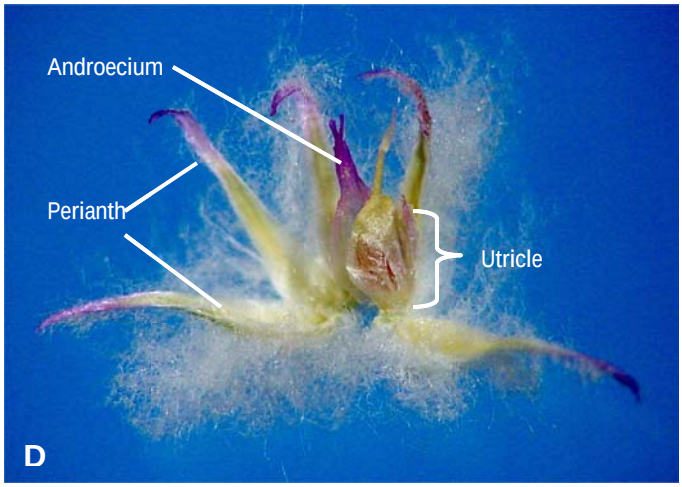


Figure 4.38.3. *Gomphrena globosa* (globe amaranth). A – Inflorescence. B – Flower subtended by bracts. C – Flower with hairy perianth; tip of androecium visible (purple). D – Perianth opened and tubular androecium partially removed to expose the utricle containing a single seed. E – Utricle.

The fruit in *Bassia* is surrounded by a persistent five-parted winged perianth that is thin and fragile. For *Bassia scoparia* 'Trichophylla' (summer cypress, Mexican fire bush) the seed units are visually inspected. Pure seed includes all intact fruits whether or not a seed is present, as well as broken fruits larger than one-half the original size unless no seed is present. Although *B. prostrata* (forage kochia) has the same basic definition for the seed unit, the purity analysis requires an additional step. Seed units that are retained by a 1-mm square-hole sieve when shaken for 30 seconds are classified as pure seed, while seed units that fall through the sieve are classified as inert matter. Seed units that fall through the 1 mm sieve have been shown to be of little planting value (Allen, et al. 1986). After sieving, the extraneous inert matter (e.g., stems, leaves, etc.) must be removed from the portion that did not fall through the sieve. This material is combined with the inert matter that falls through the sieve to determine the percentage of inert matter.

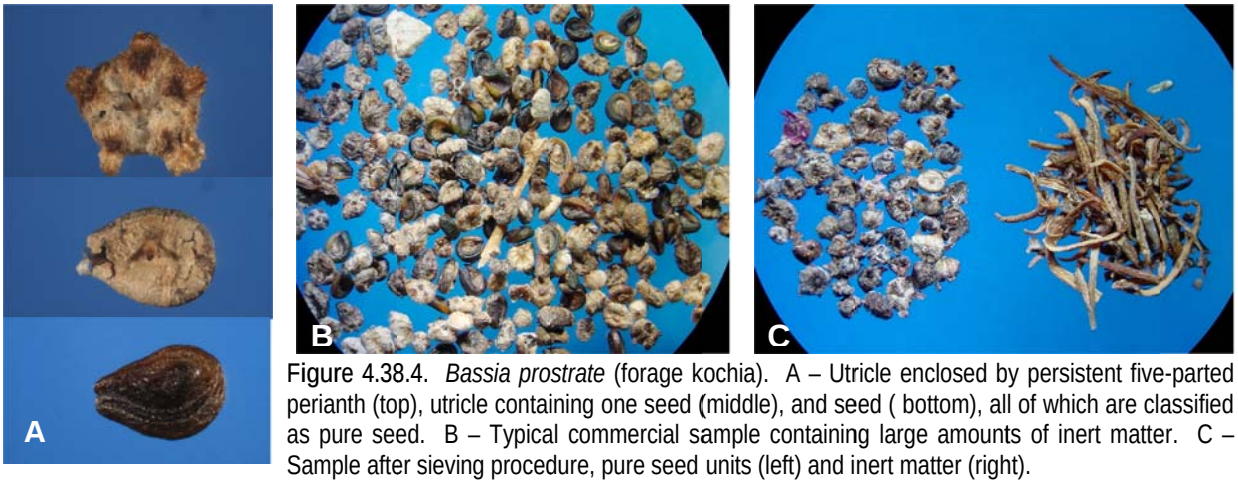


Figure 4.38.4. *Bassia prostrate* (forage kochia). A – Utricle enclosed by persistent five-parted perianth (top), utricle containing one seed (middle), and seed (bottom), all of which are classified as pure seed. B – Typical commercial sample containing large amounts of inert matter. C – Sample after sieving procedure, pure seed units (left) and inert matter (right).

References

Allen, P. S., S. E. Meyer, G. R. Wilson, T. D. Davis, J. N. Davis, R. Stevens, and K. Jorgensen. AOSA rule change proposal 6: Additional of Kochia prostrate, forage kochia, to the Rules. AOSA News Letter Vol. 60(1):19-20.

Booth, D. T. 2008. *Krascheninnikovia lanata* (Pursh) A.D.J. Meeuse & Smit, winterfat. In: Bonner, F. T. and R. P. Karrfalt (eds.). The woody plant seed manual. Agriculture Handbook 727. United States Department of Agriculture – Forest Service. 626-630 p.

Kitchen, S. G. and S. B Monsen. 2008. *Kochia* Roth, kochia. In: Bonner, F. T. and R. P. Karrfalt (eds.). The woody plant seed manual. Agriculture Handbook 727. United States Department of Agriculture – Forest Service. 620 - 623 p.

Martin, A. C. 1946. The comparative internal morphology of seeds. The American Midland Naturalist, Vol. 36(3):513-660.

Meyer, S. E. 2008. *Atriplex* L., saltbush. In: Bonner, F. T. and R. P. Karrfalt (eds.). The woody plant seed manual. Agriculture Handbook 727. United States Department of Agriculture – Forest Service. 283 - 290 p.

2011 Rules Change Proposal 15

Purpose: To modify Pure Seed Unit 50 to include the persistent enclosing bract of the ray achene for *Layia* spp.

Present and proposed rule:

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
50	Intact achene, with or without one or more of the following structures: beak, bristle, hairs, pappus, wing, or firmly attached floral remnants, provided a true seed with some degree of embryo development can be detected (either by slight pressure or by examination over light). Piece of broken achene larger than one-half of the original size, unless no seed is present. Seed, with or without pericarp/seed coat. Piece of broken seed, with or without pericarp/seed coat, larger than one-half the original size. Special consideration: • For *Layia*, the persistent bract enclosing the ray achene is part of the pure seed unit.

Harmonization and impact statement: This genus is not covered by the Federal Seed Act or the Canadian Food Inspection Agency Methods, Procedures for Testing Seeds. The International Seed Testing Association Rules for Seed Testing also does not include this genus; however, under Pure Seed Definition 4, to which many members of Asteraceae are assigned, a variety of structures may remain attached to the achene, including bracts.

Supporting evidence: *Layia* is a genus of 14 species native to western North America. The fruits of *Layia* are of two types: (1) pappose disk achenes, and (2) epappose ray achenes. The ray achenes of *Layia* are enclosed by a persistent bract. Removing this bract during a purity analysis can be time consuming and may cause damage to the achene. This kind of seed is sold commercially with the bract attached. It is possible to determine if the ray achene enclosed by the persistent bract contains an embryo by gentle application of pressure.

Submitted by: Deborah Meyer, Purity Subcommittee Chair, California Department of Food and Agriculture, (916) 262-1137, dmeyer@cdfa.ca.gov.

Date Submitted: October 15, 2010

Layia platyglossa, tidy tips daisy, the two types of achenes include disk achenes with attached pappi and epappose ray achenes enclosed in persistent bracts.

Layia platyglossa, ray achenes enclosed in persistent bracts (left) and with bract removed (right).

2011 Rules Change Proposal 16

Purpose: To reassign the pure seed unit for *Hibiscus* spp. from PSU 1 to PSU 8.

Present rule:

Table 2.4 Weights for working samples.

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
1	*Hibiscus spp.* hibiscus	-	-	-	-

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
1	Seed, with or without seed coat. Piece of broken seed, with or without seed coat, larger than one-half the original size. Special considerations: * Seeds of Cucurbitaceae and Solanaceae whether or not they are filled.

Proposed Rule:

Table 2.4 Weights for working samples.

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
8	*Hibiscus spp.* hibiscus	-	-	-	-

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
8	Seed, with or without seed coat, seed coat with or without hairs. Piece of broken seed, with or without seed coat, larger than one-half the original size.

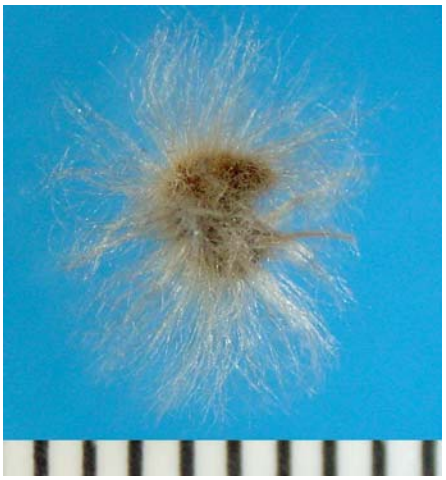
Harmonization and impact statement: This genus is not covered by the Federal Seed Act or the Canadian Food Inspection Agency Methods, Procedures for

Testing Seeds. The International Seed Testing Association Rules for Seed Testing includes only *Hibiscus cannabinus* (kenaf or rose mallow) and *H. trionum* (flower-of-an-hour) neither of which have hairy seed coats; therefore, this proposal will not affect harmonization between the AOSA and ISTA rules.

Supporting evidence: The seeds of some species of *Hibiscus* have hairy seed coats. Currently all species of *Hibiscus* are assigned to PSU1. By definition, those with hairy seed coats would need to have the hairs removed. Since this is impractical and undesirable, it would be best to assign Hibiscus spp. to PSU 8 in which hairs on the seed coat are considered part of the pure seed unit. *H. cannabinus* is the only species of Hibiscus with a separate listing in Table 2A. Since seeds of this species are not hairy this species should remain under PSU1.

Submitted by: Deborah Meyer, Purity Subcommittee Chair, California Department of Food and Agriculture, (916) 262-1137, dmeyer@cdfa.ca.gov.

Date Submitted: October 15, 2010



Hibiscus denudatus, paleface, seed

Hibiscus syriacus, rose-of-Sharon, seed

2011 Rules Change Proposal 17

Purpose: To reassign the pure seed unit for *Bouteloua dactyloides* (buffalograss) and *Pennisetum ciliare* (buffelgrass) marketed as hulled caryopses from PSU 18 and 19 to PSU 25.

Present rule:

Table 2.4 Weights for working samples.

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
19	*Bouteloua dactyloides* (Nutt.) Columbus buffalograss (burs) (caryopses)	 20 3	 200 30	 110 740	 3,120 20,960
18	*Pennisetum ciliare* (L.) Link buffelgrass (caryopses) (fascicles)	 2 6	 20 60	 1,940 357	 55,095 10,120

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
18	Fascicle, consisting of bristles and spikelets, provided a caryopsis with some degree of endosperm development can be detected (either by slight pressure or by examination over light). Caryopsis or piece of broken caryopsis larger than one-half of the original size.
19	Intact bur, whether or not a caryopsis is present. Piece of bur larger than one-half the original size unless no caryopsis is present. Caryopsis or piece of broken caryopsis larger than one-half of the original size.

Proposed Rule:

Table 2.4 Weights for working samples.

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
19 25	*Bouteloua dactyloides* (Nutt.) Columbus buffalograss (burs) (caryopses)	 20 3	 200 30	 110 740	 3,120 20,960
25 18	*Pennisetum ciliare* (L.) Link buffelgrass (caryopses) (fascicles)	 2 6	 20 60	 1,940 357	 55,095 10,120

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
18	Fascicle, consisting of bristles and spikelets, provided a caryopsis with some degree of endosperm development can be detected (either by slight pressure or by examination over light). Caryopsis or piece of broken caryopsis larger than one-half of the original size.
19	Intact bur, whether or not a caryopsis is present. Piece of bur larger than one-half the original size unless no caryopsis is present. Caryopsis or piece of broken caryopsis larger than one-half of the original size.
25	Caryopsis or piece of broken caryopsis larger than one-half of the original size.

Harmonization and impact statement: These species are covered by the Federal Seed Act and have separate working weights for hulled caryopses and burs or fascicles; therefore, when testing a sample from a lot marketed as hulled caryopses the seed unit is the caryopsis or broken caryopsis fragment larger than one-half the original size because the caryopsis is the structure regarded as a seed in planting practices and in commercial channels (Sec. 201.47a).

The International Seed Testing Association Rules for Seed Testing does not include *Bouteloua dactyloides*; therefore, harmonization is not a consideration. The ISTA Rules do include *Pennisetum ciliare* (*Cenchrus ciliaris*), but only provide working weights for the fascicles. No mention is made regarding what to do if a seed lot is marketed as hulled caryopses. Since AOSA Rules include the same working sample weights for the fascicles of this species, AOSA and ISTA are already harmonized for testing of fascicles of this species.

No reference is made to either species in the Canadian Food Inspection Agency Methods, Procedures for Testing Seeds.

Supporting evidence: When *Bouteloua dactyloides* (buffalograss) and *Pennisetum ciliare* (buffelgrass) are marketed as hulled caryopses the assumption is the caryopsis is the seed unit to be planted. Working weights in Table 2A for these species include two options, one for fascicles or burs and one for hulled caryopses. Other kinds in Table 2A, such as *Cynodon dactylon* (bermudagrass) and *Pleuraphis jamesii* (galleta grass), are treated with duel PSU assignments to accommodate the multiple formats in which the seed is marketed. When testing a sample from a seed lot that is marketed as hulled caryopses, if an occasional bur or fascicle is found in the sample the caryopses would be removed and classified as pure seed and the remaining bur or fascicle structures would be classified as inert matter.

Submitted by: Deborah Meyer, Purity Subcommittee Chair, California Department of Food and Agriculture, (916) 262-1137, dmeyer@cdfa.ca.gov.

Date Submitted: October 15, 2010

2011 Rules Change Proposal 18

Purpose: To correct the improper assignment of the pure seed unit for *Bothriochloa ischaemum* (yellow bluestem). The assignment will change from PSU 14 to PSU 15.

Present rule:

Table 2.4 Weights for working samples.

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
14	*Bothriochloa ischaemum (L.) Keng* yellow bluestem	1	10	1,945	55,170

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
14	Multiple floret spikelet, multiple floret, or floret, with or without awn(s), provided a caryopsis with some degree of endosperm development can be detected (either by slight pressure or by examination over light). Caryopsis or piece of broken caryopsis larger than one-half of the original size.

Proposed Rule:

Table 2.4 Weights for working samples.

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
15	*Bothriochloa ischaemum (L.) Keng* yellow bluestem	1	10	1,945	55,170

Table 3A. Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
15	Spikelet with or without attached rachis segment (also see rame internode in glossary), pedicel and sterile spikelet, with or without awn(s), provided a caryopsis with some degree of endosperm development can be detected (either by slight pressure or by examination over light). Caryopsis or piece of broken caryopsis larger than one-half of the original size.

Harmonization and impact statement: The Federal Seed Act and the International Seed Testing Association Rules for Seed Testing includes this species with pure seed units that include spikelets with attached rachis segment and pedicel. The proposed change will bring the AOSA, FSA, and ISTA rules into harmony.
This species is not specifically mentioned in the Canadian Food Inspection Agency Methods, Procedures for Testing Seeds.

Supporting evidence: Clearly *Bothriochloa ischaemum,* yellow bluestem, was mistakenly assigned to PSU 14 and should be placed with other similarly structured grasses that are members of the Andropogoneae. In this tribe of grasses the rachis segment or rame internode that supports the next most distal sessile spikelet and the pedicel that supports the pedicellate spikelet remain attached to the sessile spikelet. Disarticulation occurs at the base of the sessile spikelet and the sessile and pedicellate spikelet (sterile spikelet) fall as a pair.

Submitted by: Deborah Meyer, Purity Subcommittee Chair, California Department of Food and Agriculture, (916) 262-1137, dmeyer@cdfa.ca.gov.

Date Submitted: October 15, 2010

Bothriochloa ischaemum, yellow bluestem. Spikelets

Photograph by Jose Hernandez @ USDA-NRCS PLANTS Database

2011 Rules Change Proposal 19 Amended

Purpose of Proposal: To add purity and germination methods for *Diplotaxis* spp. (wallrocket, wild arugula) to the AOSA Rules.

Present Rule:
AOSA Rules for Testing Seeds Volume 1. Principles and Procedures: New rule

AOSA Rules for Testing Seeds Volume 3. Uniform Classification of Weed and Crop Seeds -- Uniform Classification Sorted by Scientific Name:

NOMEN #	SCIENTIFIC NAME	COMMON NAME	FAMILY	SPP. CLASS	CONTAMINATING CLASSIFICATION						
					A	F	H	R	S	T	V
317046	*Diplotaxis* spp.	wallrocket	Brassicaceae	W	W	W	W	W	W	W	W
316651	*Diplotaxis tenuifolia* (L.)DC.	wallrocket	Brassicaceae	W	W	W	W	W	W	W	W

Proposed Rule:
AOSA Rules for Testing Seeds Volume 1. Principles and Procedures:

Table 2A. Weights for working samples.

Pure Seed Unit #	Kind of seed	Minimum weight for purity analysis	Minimum weight for noxious-weed seed or bulk examination	Approximate number of seeds per gram	Approximate number of seeds per ounce
		Grams	Grams	Number	Number
2	*Diplotaxis* spp. wallrocket; wild arugula, wild rocket	0.7	7	3,890	110,270

Table 6A. Methods of testing for laboratory germination.

Kind of Seed	Substrata[a]	Tempera-ture (°C)	First count (days)	Final count (days)	Specific requirements and notes	Fresh and dormant seed
Diplotaxis spp. wallrocket, wild arugula, wild rocket	B	20-30	4	10	Light; KNO3	

AOSA Rules for Testing Seeds Volume 3. Uniform Classification of Weed and Crop Seeds -- Uniform Classification Sorted by Scientific Name:

NOMEN #	SCIENTIFIC NAME	COMMON NAME	FAMILY	SPP. CLASS	CONTAMINATING CLASSIFICATION						
					A	F	H	R	S	T	V
317046	*Diplotaxis* spp.	wallrocket; arugula, wild, rocket, wild	Brassicaceae	V	W	W	W	W	W	W	W
316651	*Diplotaxis tenuifolia* (L.)DC.	wallrocket; arugula, wild, rocket, wild	Brassicaceae	V	W	W	W	W	W	W	W

Harmonization and Impact Statement: No members in this genus are included in the ISTA rules, Canadian methods, or the Federal Seed Act.

Supporting Evidence: *Diplotaxis* is a genus in the Brassicaceae (mustard family) consisting of about 35 species of annual and perennial herbs native to the Mediterranean region of Central Europe to northwest India. At least two species (*D. muralis* and *D. tenuifolia*) are increasingly used as ingredients for green salads; at Ransom Seed Lab we have received samples for testing from at least six different seed companies over the past few years, indicating the need for standardized testing methods. Various common names have been used for these species, including wild arugula, wallrocket, wild rocket, rucola, wasabi rocket, and white rocket. For simplicity, the authors have limited the common names to “wallrocket” (already listed this way in the AOSA Rules Volume3) and “wild arugula.”

Seed counts were done by hand-counting 1000 seeds from 10 different lots submitted as commercial samples.

***Diplotaxis* Seed Count:**

Sample #	1000 seeds (g)	# seeds/gram	# seeds/ounce	2500 seeds (g)	25,000 seeds (g)
4435	.2614	3826	108,467	.65	6.53
6577	.2273	4399	124,712	.57	5.68
6578	.2920	3425	97,099	.73	7.30
6579	.2747	3640	103,194	.69	6.87
6580	.2843	3517	99,707	.71	7.11
7202	.2281	4384	124,286	.57	5.70
7865	.2278	4390	124,457	.57	5.70
10050	.2512	3981	112,861	.63	6.28
11085	.2786	3589	101,748	.70	6.97

11086	.2670	3745	106,171	.67	6.68
	Average	**3890**	**110,270**	**.65**	**6.48**

From the above data the proper minimum purity weight would be .0.7 grams and the minimum noxious weed exam would be 7 grams.

In order to determine the best germination method for *Diplotaxis* spp, a series of laboratory tests were run over several months on commercial samples received from several different seed companies. All tests were done on 4 replicates of 100 seeds planted on top of blotters in light.

Series 1:

Sample #	10-day germ at 20-30 C	10-day germ at 20 C
4646	89	47
4647	85	50
4648	89	42
6578	87	67
6579	90	69
6580	84	62

Series 2:

Sample #	10-day germ at 20-30 C	10-day germ at 27 C
6577	42	23
6578	87	76
6579	90	81
6580	84	80
7202	88	69
7865	87	87
10500	90	74

Series 3:

Sample #	Germination results (%) after 10 days:			
	20-30 C H_20	20-30 C KNO_3	20 C H_20	Prechill, GA_3
4649	81	**94**	--	94
7202	87	**88**	25	88
9346	80	**81**	52	81
9497	92	**90**	57	83
9693	80	**75**	45	73
11085	75	**90**	26	89
11086	84	**88**	52	84

From this series of laboratory tests, it was found that 20-30 C was the best temperature. However, some lots exhibited dormancy. Further testing revealed

that the combination of 20-30C and 0.2% KNO_3 broke the remaining dormancy. This was also accomplished by prechilling with 400 ppm GA_3 and transferring to 20-30 C. First counts of 4 days are recommended, with a final count of 10 days sufficient for maximum potential germination.

References:

Bailey, LH (ed.) 1976. Hortus Third – A Concise Dictionary of Plants Cultivated in the United States and Canada. Macmillan Publishing Company.

Hickman, James (ed.). 1993. The Jepson Manual: Higher Plants of California. University of California Press.

Morales, M. and J. Janick. 2002. Arugula: A promising specialty leaf vegetable. p. 418–423. In: J. Janick and A. Whipkey (eds.), Trends in new crops and new uses. ASHS Press, Alexandria, VA.
http://www.hort.purdue.edu/newcrop/ncnu02/v5-418.html

Condor Seed Production website: http://www.condorseed.com/seed_herbs.htm

Enza Zaden website: http://enzazaden.com/Products/herbs/rucola

Seeds of Change website:
http://www.seedsofchange.com/garden_center/product_details.aspx?item_no=PS17107

Submitted By:

Aleta Meyr, R.S.T
Sue Alvarez, R.S.T.

Ransom Seed Laboratory
P.O. Box 300
Carpinteria, California 93014-0300
Phone: 805-684-3427
Fax: 805-684-4157
Email: RansomSL@silcom.com

Date Submitted: October 15, 2010

2011 Rules Change Proposal 20

Purpose of Proposal: To change the first count (days) requirement from 6 to 10 for Vegetable and Ornamental Pepper *(Capsicum spp.)*. First count evaluation on 6 days does not allow enough time for seedling development. All essential parts are not visible for accurate evaluation.

Present Rule:

Table 6A. Methods of testing for laboratory germination.

Kind of Seed	Substrata	Tempera-ture (°C)	First count (days)	Final count (days)	Specific requirements and notes	Fresh and dormant seed
Capsicum spp. **vegetable and ornamental pepper**	**T, B, TB, RB, P**	**20-30**	**6**	**14**		**Light and KNO_3. See footnotes b and c for ornamental varieties**

Proposed Rule:

Table 6A. Methods of testing for laboratory germination.

Kind of Seed	Substrata	Tempera-ture (°C)	First count (days)	Final count (days)	Specific requirements and notes	Fresh and dormant seed
Capsicum spp. **vegetable and ornamental pepper**	**T, B, TB, RB, P**	**20-30**	**10**	**14**		**Light and KNO_3. See footnotes b and c for ornamental varieties**

Harmonization: The proposed rule for Vegetable and Ornamental Pepper *(Capsicum spp.)* does not harmonize with the Federal Seed Act (first count 6 days), the Canadian Methods & Procedures (first count 7 days) and the International Seed Testing Association Rules (first count 7 days). It is stated in ISTA Rules Chapter 5, Section 5.6.4, Duration of Test; the time of the first count is approximate but must be sufficient to permit the seedlings to reach a stage of development which allows for accurate evaluation.

Supporting Evidence: See Region IV Southwest 2009-2010 Referee on the First Evaluation of Vegetable and Ornamental Pepper *(Capsicum spp.)* Seedlings (included in appendix). This referee was proposed because the first evaluation (6 days) in the AOSA Rules Table 6A for Vegetable and Ornamental Pepper *(Capsicum spp.)* may not give sufficient time for seedling development.

Additional Supporting Evidence: MD Seed Analysis (an independent seed testing lab) conducted germination tests on over 200 vegetable pepper *(Capsicum spp.)* samples according to ISTA Rules in 2009 and over 220 vegetable pepper *(Capsicum spp.)* samples according to ISTA Rules through August 2010. The first evaluation (7 days), the 10 day evaluation and the final count (14 days) were tracked on 77 vegetable pepper *(Capsicum spp.)* samples which included 17 different varieties. The counts for each variety were averaged. The results are shown in Table 1 below.

Table 1.

Capsicum spp. **Pepper variety**	**First Count 7 day**	**10 day**	**Final Count 14 day**
A	5	78	96
B	16	75	95
C	2	82	97
D	10	80	95
E	4	81	96
F	0	96	98
G	0	83	91
H	1	89	95
I	0	58	94
J	24	65	96
K	0	95	97
L	0	86	90
M	0	70	89
N	0	64	92
O	0	96	98
P	0	37	88
Q	3	82	93
Mean	**3.8**	**77.5**	**94.1**

Summary of Supporting Evidence: The rule proposal to change first evaluation to 10 days for Vegetable and Ornamental Pepper *(Capsicum spp.)* allows adequate time for all essential structures to develop. Evaluation will be more consistent among analysts (standard) and more accurate by seeing all essential parts. The evaluation will be faster because seed coats will not need to be removed which eliminates the possibility of seedling damage. Also first evaluation on 10 days eliminates the need for an intermediate count saving time on evaluating the germination test.

Reference: Seedling Evaluation of Pepper (*Capsicum spp.*) SOLANACEAE
A Guide to Seedling Evaluation for Use in Seed Germination
Revised April 2005
Prepared by:
Paul S. Peterson
California Department of Food & Agriculture
Sacramento, CA
Elaine Harris
California Department of Food & Agriculture
Sacramento, CA

Submitted By:
Donna Grubisic
MD Seed Analysis, Inc.
123 N. Salinas St.
Santa Barbara, CA 93103
Phone 805-962-0739
Fax 805-962-0927
mdseedanalysis@verizon.net

Date Submitted: October 15, 2010

Appendix 1 for Proposal 20 Supporting Evidence

Region IV Southwest 2009-2010 Referee on the First Evaluation of Vegetable and Ornamental Pepper *(Capsicum spp.)* Seedlings

This referee was proposed because the first evaluation (6 days) in the AOSA Rules Table 6A for Vegetable and Ornamental Pepper *(Capsicum spp.)* may not give sufficient time for seedling development. All essential parts of the pepper seedling are not visible on 6 days. Evaluation on 6 days is not possible without removing the seed coat, adding additional time to the evaluation plus possible seedling damage.

The purpose of the referee was to compare pepper seedling development on 6 days (AOSA Rules) to seedling development on 10 days.

A survey was first sent out that included questions on pepper *(Capsicum spp.)* germination testing. Nineteen (19) analysts from eleven (11) labs participated in the survey. Below are the questions asked as well as the responses.

1) Does your lab perform germination tests on pepper *(Capsicum spp.)* seed?

All analysts do germination testing on pepper *(Capsicum spp.)* seeds.

2) How many pepper *(Capsicum sp.)* samples do you perform germination tests on per year?

11 analysts test 1 – 25 pepper *(Capsicum spp.)* samples per year.
5 analysts test 25 – 100 pepper *(Capsicum spp.)* samples per year.
3 analysts test 100 – more pepper *(Capsicum spp.)* samples per year.

3) What method do you use for germination tests on pepper *(Capsicum spp.)* seeds?

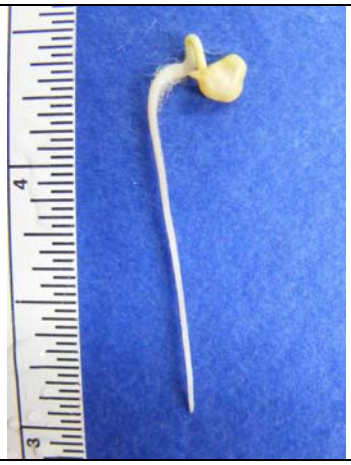
11 analysts used testing method – RT, 20-30ºC, water
4 analysts used testing method – RT, 20-30ºC, KNO3
2 analysts used testing method – RT, 20-30ºC, light, KNO3
2 analysts used testing method – Petri dish, 20-30ºC, water

4) When do you perform the first evaluation on pepper *(Capsicum spp.)* germination tests?

2 analysts first evaluation 10 days
1 analyst first evaluation 9 days
1 analyst first evaluation 7 – 8 days
2 analysts first evaluation 7 days
12 analysts first evaluation 6 days
1 analyst first evaluation 6 or 8 days

5) Pictures of pepper *(Capsicum spp.)* seedlings from germination tests on 6 days and 10 days were included. The analyst was asked if the pictured seedlings can be evaluated for the first count of 6 days or 10 days according to the AOSA Seedling Evaluation Handbook.

Sample	Picture	Analysts' response
Sample A: 6 day seedlings		• **5 analysts: yes** can be evaluated – healthy, developing well •**14 analysts: no** can not be evaluated – not able to view cotyledons
Sample A: 10 day seedlings		• **17 analysts: yes** can be evaluated – all essential parts visible • **2 analysts: no** can not be evaluated – seed coat still attached
Sample B: 6 day seedlings		• **5 analysts: yes** can be evaluated - all parts present • **1 analyst: yes?** can be evaluated- remove seed coat • **12 analysts: no** can not be evaluated – seed coats attached, cotyledons not visible • **1 analyst: yes** on 3 sprouts (visible cotyledons) and **no** on 4 sprouts (cotyledons not visible)
Sample C: 6 day seedlings		• **10 analysts: yes** can be evaluated – 5 analysts remove seed coat to evaluate normal • **1 analyst: yes?** can be evaluated - remove seed coat • **8 analysts: no** can not be evaluated – cotyledons not visible

Sample D: 10 day seedling		• **18 analysts: yes** can be evaluated – all essential parts visible • **1 analyst: no** can not be evaluated- seed coat still attached
Sample D: 6 day seedling		• **10 analysts: yes** can be evaluated – 5 analysts remove seed coat to evaluate normal • **1 analyst: yes?** can be evaluated removing seed coat • **8 analysts: no** can not be evaluated- hypocotyl short, cotyledons not visible

Next, after the survey was returned, three lots of pepper *(Capsicum spp.)* seeds were sent to 19 analysts (9 Labs) that were supplied by Laurie Conradson, RST of Harris Moran Company. The analysts were asked to perform a germination test of 400 seeds on each lot according to the testing methods described in the AOSA Rules. Of the 4 replicates, #1 and #2 were to be evaluated on 6 days and replicates #3 and #4 were to be evaluated on 10 days. All 4 replicates were to be finalized on 14 days. The Tables 1 – 3 below show the averaged results of the germination tests from 16 analysts that returned their data.

Table 1.

First Evaluation – 6 days			
Analyst	**Sample A**	**Sample B**	**Sample C**
1	81	89	77
2	81	92	84
3	69	93	77
4	86	93	83
5	28	80	38
6	0	0	0
7	0	0	0
8	0	0	0
9	0	0	0
10	0	0	0
11	1	0	1
12	0	0	0
13	0	0	0
14	0	0	0
15	8	12	42
16	6	4	32
Mean	**22.5**	**28.9**	**27.1**
sd	**34.7**	**42.3**	**34.9**

Table 2.

First Evaluation – 10 days			
Analyst	**Sample A**	**Sample B**	**Sample C**
1	91	98	80
2	92	94	89
3	91	100	87
4	89	93	89
5	93	100	70
6	84	89	82
7	79	86	82
8	48	49	72
9	67	76	81
10	34	25	67
11	61	91	54
12	35	39	23
13	86	94	91
14	76	91	84
15	95	97	88
16	91	97	83
Mean	**75.8**	**82.4**	**76.4**
sd	**20.7**	**23.4**	**17.3**

Table 3.

Final Evaluation – 14 days			
Analyst	**Sample A**	**Sample B**	**Sample C**
1	93	99	87
2	93	96	89
3	91	99	91
4	92	97	90
5	91	99	71
6	89	97	85
7	94	99	89
8	93	96	88
9	92	96	88
10	93	96	92
11	95	99	87
12	88	97	76
13	93	99	93
14	93	99	92
15	95	99	91
16	92	97	89
Mean	**92.3**	**97.8**	**87.4**
sd	**1.9**	**1.3**	**5.9**

The data from the survey indicated that the pepper *(Capsicum spp.)* seedlings showed all essential parts for evaluation on 10 days, but on 6 days all essential parts were not visible. The germination tests indicated higher percentages of first evaluation on 10 days compared to 6 days.

Table 4 below compares the percentages between germination results on 6 days and 10 day first counts obtained from the referee. This table shows that the 6 day evaluation results are significantly lower than the 10 day evaluation results. Table 4 also shows that the SD, minimum, maximum and range are greater for 6 day evaluations than 10 day, meaning there is more variation and less consistency when analysts evaluate after only 6 days.

Table 4.

		Evaluation Days – First Count	
		6 days	**10 days**
Sample A	Minimum	0	34
	Maximum	86	95
	Range	86	61
	Mean	22.5	75.8
	SD	34.7	20.7

Table 4. (cont.)			
Sample B	Minimum	0	25
	Maximum	93	100
	Range	93	75
	Mean	28.9	82.4
	SD	42.3	23.4
Sample C	Minimum	0	23
	Maximum	84	91
	Range	84	68
	Mean	27.1	76.4
	SD	34.9	17.3

Table 5 below shows the average of the replicates from the 6 day evaluation and their 14 day final as well as the average of the replicates for the 10 day evaluation and their 14 day final from each analyst who participated in the referee. The 6 and 10 day first evaluations show similar 14 day final results (in tolerance) indicating that changing the first count from 6 to 10 has no effect on final germination.

Table 5.

Analyst 1	**Sample 1**	**Sample 2**	**Sample 3**
6day / 14 day	81 / 95	89 / 100	77 / 92
10 day / 14 day	91 / 92	98 / 99	80 / 82
Analyst 2			
6 day / 14 day	81 / 94	92 / 97	84 / 88
10 day / 14 day	92 / 93	94 / 95	89 / 89
Analyst 3			
6day / 14 day	69 / 91	93 / 100	77 / 91
10 day / 14 day	91 / 91	100 / 100	87 / 91
Analyst 4			
6 day / 14 day	86 / 94	93 / 99	83 / 88
10 day / 14 day	89 / 90	93 / 95	89 / 92
Analyst 5			
6 day / 14 day	28 / 88	80 / 98	38 / 71
10 day / 14 day	93 / 94	100 / 100	70 / 72
Analyst 6			
6 day / 14 day	0 / 92	0 / 97	0 / 86
10 day / 14 day	84 / 86	89 / 96	82 / 84

Table 5. (cont.)			
Analyst 7	**Sample 1**	**Sample 2**	**Sample 3**
6 day / 14 day	0 / 94	0 / 99	0 / 91
10 day / 14 day	79 / 94	86 / 99	82 / 89
Analyst 8			
6 day / 14 day	0 / 93	0 / 96	0 / 88
10 day / 14 day	48 / 93	49 / 96	72 / 92
Analyst 9			
6 day / 14 day	0 / 92	0 / 96	0 / 88
10 day / 14 day	67 / 92	76 / 96	81 / 88
Analyst 10			
6 day / 14 day	0 / 93	0 / 96	0 / 92
10 day / 14 day	34 / 93	25 / 96	67 / 92
Analyst 11			
6 day / 14 day	1 / 93	0 / 99	1 / 89
10 day / 14 day	61 / 96	91 / 99	54 / 85
Analyst 12			
6 day / 14 day	0 / 89	0 / 97	0 / 79
10 day / 14 day	35 / 87	39 / 97	23 / 73
Analyst 13			
6 day / 14 day	0 / 97	0 / 99	0 / 92
10 day / 14 day	86 / 89	94 / 99	91 / 94
Analyst 14			
6 day / 14 day	0 / 93	0 / 99	0 / 93
10 day / 14 day	76 / 93	91 / 99	84 / 91
Analyst 15			
6 day / 14 day	8 / 94	12 / 99	42 / 91
10 day / 14 day	95 / 96	97 / 99	88/ 91
Analyst 16			
6 day / 14 day	6 / 93	4 / 97	32 / 88
10 day/ 14 day	91 / 91	97 / 97	83 / 90
Mean – Final Germination			
6 / 14 day *	92.8	98.0	87.9
10 / 14 day *	91.9	97.6	87.2

***** 6 / 14 and 10 / 14 refer to final germination results following a first count on day 6 and 10, respectively.

2011 Rules Change Proposal 21 Amended

Purpose of Proposal: To clarify and add details to the rules on calculating percentage germination when multiple germination tests are made.

Present Rule:

6.7 Calculation of percentage germination

b. *When more than one test is made*, the results of such tests or retests that fall within one tolerance range, or tests by alternate methods, shall be averaged and reported as the percentage germination. When different official methods are employed, and the highest result is not within tolerance of the next lower, the higher percentage shall be reported. When retests are required because of out of tolerance replicates and are satisfactory (section 6.6 a) but out of tolerance with the original test, a third test must be conducted and the average of the tests that are within tolerance reported (if the result of the third test falls between the first two, and is in tolerance with both, report the average of the three tests). When retests are required for other reasons (sections 6.6 b, c, d and e) and are satisfactory but out of tolerance with the original test, the higher percentage shall be reported.

c. *When paired tests are made*, percentage germination shall be reported for each test condition.

d. For *Nassella viridula*, *Penstemon penlandii*, *Penstemon eatonii*, *Penstemon* spp., and *Hesperostipa comata*, report results of Method 2 (see Table 6A and sections 6.8 k, m, p and q), as percentage germination. If the number in Method 2 is less than in Method 1, subtract results of Method 2 from Method 1 and report the difference as dormant seed percentage.

Proposed Rule:

6.7 Calculation of percentage germination

b. *When more than one test is made on the same submitted sample*

1) If more than one test is made using the same official method, the results of such tests or retests that fall within one tolerance range shall be averaged and reported as the percentage germination. If two tests are made and are within tolerance, average those results. If results of the two tests are out of tolerance, a third test is made and results of the two tests that are within tolerance are averaged and reported as percentage germination. For example, if germination results of the first test are 95%, second test 81%, and third test79%, percentage germination is reported as 80%, the average of the second and third tests. If the first two tests are not within tolerance and result of a third test falls between the first two, and is in tolerance with both, report the average of the

three tests. For example, if germination results of the first test are 92%, the second 80%, and the third 87%, percentage germination is reported as 86%, the average of the three test results. If none of the first three tests are within tolerance, additional tests are made until at least two are within tolerance.

2) When a retest is required because of out of tolerance replicates, and the second test results are in tolerance with the original test, the average of the two test results shall be reported, if the replicates of the second test are within tolerance. When a retest is required because of out of tolerance replicates and the second test results are out of tolerance with the original test, a third test must be conducted and the average of the tests that are within tolerance reported. If the result of the third test falls between the first two, and is in tolerance with both, report the average of the three tests. When a retest is required because of out of tolerance replicates and the replicates of the second test are out of tolerance, additional tests are made until a retest is satisfactory (replicates within tolerance) and within tolerance with one of the previous tests; results of those tests are averaged and reported as percentage germination.
3) When different official methods are employed, results of the different methods cannot be averaged. The highest percentage germination shall be reported, with the option of reporting results of other methods. Conditions and testing dates for all reported methods, in accordance with existing rules, shall be reported.
4) When retests are required for reasons other than being out of tolerance (sections 6.6 b, c and d) and are satisfactory (replicates within tolerance) and within tolerance with the original test, the two test results shall be averaged and reported as the percentage germination. When retests are required for other reasons (sections 6.6 b, c and d) and are satisfactory but out of tolerance with the original test, the higher percentage germination shall be reported.

c. *When paired tests are made,* percentage germination shall be reported for each test condition. For *Pennisetum ciliare*, *Cichorium endivia*, *Bromus catharticus* and *Securigera varia*, when a second seed sample is germinated as described in sections 6.8 d, f, g and o, respectively, using 400 seeds for each test condition, the two test results are to be treated as paired tests and the percentage germination shall be reported for each test condition.

d. For *Nassella viridula*, *Penstemon penlandii*, *Penstemon eatonii*, *Penstemon* spp., and *Hesperostipa comata*, report results of Method 2 (see Table 6A and sections 6.8 k, m, p and q), as percentage germination. If the number in Method 2 is less than in Method 1, subtract results of Method 2 from Method 1 and report the difference as dormant seed percentage.

Harmonization and Impact Statement: This proposal serves as a rule clarification, providing additional details on reporting multiple test results under different situations. The proposal improves harmonization with ISTA rules (section 5.7 e) by describing similar reporting methods for germination tests when two or more replicates are out of tolerance. Neither the Federal Seed Act nor the Canadian Methods and Procedures include detailed descriptions on how to report results of multiple germination tests. This rule clarification will ensure uniform

reporting among AOSA/SCST laboratories. The current rules are too ambiguous, leading to different interpretations and inconsistent reporting.

Supporting Evidence: Members of the Germination and Dormancy Subcommittee, in their last meeting in St. Louis, recommended that section 6.7 b be subdivided into subsections, each addressing a specific situation where multiple tests are required, and the proper way of calculating percentage germination for each.

Submitted By

Riad Baalbaki, Senior Seed Botanist
California Department of Food and Agriculture
Plant Pest Diagnostics Branch
3294 Meadowview Road
Sacramento, CA 95832-1448
Phone: (916) 262-3292
Fax: (916) 262-1190
Email: rbaalbaki@cdfa.ca.gov

Date Submitted

October 15, 2010

Revised November 10, 2010

2011 Rules Change Proposal 22 Amended

Purpose: To move Appendix 3 for determination of sample working weights for kinds not listed in Table 2A (non-mechanical seed counts) into a newly created section on non-mechanical seed counts and to clarify the appropriate use of the procedure, the source of the seed to be used for non-mechanical seed counts, and calculation of the results. No significant changes are made to the current AOSA procedure.

Present rule:

APPENDIX 3: DETERMINATION OF WEIGHT OF WORKING SAMPLES FOR KINDS NOT LISTED IN TABLE 2A

To determine the weight of the purity working sample and its corresponding noxious-weed seed working sample the Weight Determination Method will be followed (adapted from: International Seed Testing Association, Rules for Seed Testing, Chapter 10. Weight Determination).

From the working sample count out at random, by hand or with a germination counter, eight replicates, each of 100 seeds. Weigh each replicate in grams to four significant figures (see Appendix 2). With small-seeded kinds it is not necessary to weigh the replicates with greater precision than four decimal places.

Calculate the variance, standard deviation and coefficient of variation as follows:

$$\textbf{Variance} = \frac{N\sum x^2 - (\sum x)^2}{N(N-1)}$$

Where x = weight of each replicate in grams
N = number of replicates
$\sum$ = sum of

Standard deviation (s) = square root of Variance

$$\textbf{Coefficient of variation} = \frac{\text{Standard deviation}}{\text{Mean weight of 100 seeds}} \times 100$$

If the coefficient of variation does not exceed 6.0 for chaffy seeds, or 4.0 for other seeds, the result of the determination can be calculated.

If the coefficient of variation exceeds whichever of these limits is appropriate, count and weigh a further eight replicates and calculate the standard deviation for the 16 replicates. Discard any replicates that diverge from the mean by more than twice the standard deviation so calculated.

To calculate the minimum weight for purity analysis (grams): multiply the mean weight of 100 seeds by 25 (2,500 seed weight).

To calculate the minimum weight for noxious weed seed or bulk examination (grams): multiply the minimum weight for purity analysis by 10.

To calculate the approximate number of seeds per gram: divide 100 (the number of seeds) by the mean weight (of the 100 seed replicates).

To calculate the approximate number of seeds per ounce: multiply the approximate number of seeds per gram by 28.35.

For species which have small and large seeded varieties, more than one morphological seed form, or have seed weights which may vary with processing; counts should be made for each type.

Proposed rule:

SECTION 13: NON-MECHANICAL SEED COUNT AND WORKING WEIGHT DETERMINATIONS

Procedures outlined in this section may be used to determine the number of seeds per gram and the appropriate weights for purity analysis and noxious weed seed exam working samples for submitted samples of kinds with unusually small or large seeds or for kinds not listed in Table 2A. ~~Results obtained using the non-mechanical seed count methods are not intended for labeling purposes.~~

13.1 General procedures that apply to non-mechanical seed counts.

Only pure seed units shall be used in seed count and working sample weight determinations (refer to section 3.2). For kinds not listed in Table 2A select a pure seed unit definition that best describes the seed units that comprise the sample to be tested.

13.2 Source of pure seed units for non-mechanical seed count tests.

a. For kinds not listed in Table 2A.
   (1) When the kind to be tested is not listed in Table 2A divide a representative portion from the bulk in accordance with section 2.2. Select a pure seed unit definition from Table 3A that best fits the kind to be tested. If the sample is estimated to be less than 98 percent pure seed, separate the sample into two components as follows: (a) pure seed and (b) other crop seed, weed seed and inert matter. Use only pure seed units for the non-mechanical seed count.

b. For kinds listed in Table 2A.
   (1) When the minimum working weight for purity analysis is given in Table 2A, but the seed units to be tested appear to be unusually smaller or larger than typical pure seed units for the kind under consideration, a non-mechanical seed count may be used to determine an appropriate working sample weight. Divide a representative portion from the bulk in accordance with section 2.2. If the sample is estimated to be less than 98 percent pure seed, separate the sample into two components as follows: (a) pure seed and

(b) other crop seed, weed seed and inert matter. Use only pure seed units for the kind under consideration, as defined in Table 3A, for the non-mechanical seed count.

13.3 Conducting the non-mechanical seed count (adapted from: International Seed Testing Association, Rules for Seed Testing, Chapter 10. Weight Determination).

a. From the working sample count out at random without regard to size, by hand or with a germination counter, eight replicates each of 100 seed units. Weigh each replicate in grams to four significant figures (see Appendix 2). With small-seeded kinds it is not necessary to weigh the replicates with greater precision than four decimal places.

b. Calculate the variance, standard deviation and coefficient of variation as follows:

$$\textbf{Variance} = \frac{N\sum x^2 - (\sum x)^2}{N(N-1)}$$

Where x = weight of each replicate in grams
N = number of replicates
$\sum$ = sum of

Standard deviation (s) = square root of Variance

$$\textbf{Coefficient of variation} = \frac{\text{Standard deviation}}{\text{Mean weight of 100 seeds}} \times 100$$

c. If the coefficient of variation does not exceed 6.0 for chaffy seeds, or 4.0 for other seeds, the result of the determination can be calculated.

d. If the coefficient of variation exceeds whichever of these limits is appropriate, count and weigh a further eight replicates and calculate the standard deviation for the 16 replicates. Discard any replicates that diverge from the mean by more than twice the standard deviation so calculated.

13.4 Calculation of results.

a. To calculate the numbers of seeds per gram use the following formula and round results to the nearest whole number.

$$\text{Number of seeds per gram} = \frac{100}{\text{Mean weight (g) of 100 seed units}}$$

b. To determine the weight of the purity working sample and its corresponding noxious-weed seed working sample for kinds not listed in Table 2A [refer to section 2.3b(2)], or for kinds listed in Table 2A with unusually smaller or larger seed units [refer to section 2.3b(3)] the following calculations shall apply.

(1) To calculate the minimum weight for a purity analysis, multiply the mean weight of 100 seed units (grams) by 25 to determine the weight of 2,500 seed.

(2) To calculate the minimum weight for noxious weed seed or bulk examinations, multiply the minimum weight for the purity analysis (grams) by 10 to determine the weight of 25,000 seeds.

Harmonization and Impact statement: The procedure for non-mechanical seed counts was adapted from the ISTA Rules and added to the AOSA Rules several years ago. No adverse impact from this proposal is anticipated since the proposal does not seek to change the current AOSA (or ISTA) procedure, but merely serves to clarify the appropriate use of the procedure, the source of the seed for conducting the procedure, and clarifies the calculation of results.

Supporting evidence: The procedure for a non-mechanical seed count is not significantly changed from the current procedure described in Appendix 3 Determination of Weight of Working Samples for Kinds Not Listed in Table 2A. The main purpose of the proposal is to recognize that non-mechanical seed counts are conducted by many labs for a variety of reasons and to recognize the method in a section of the Rules rather than relegate it to an appendix. Data derived from non-mechanical seed counts may be used to determine the purity, noxious, and bulk sample working weights for kinds not included in Table 2A and for kinds listed in Table 2A with unusually smaller or larger seeds than typically expected. Language was added to describe the purpose of the procedure and possible sources of seed for non-mechanical seed counts. Also included is the admonition that the test results should not be used for labeling purposes. In the future, if appropriate tolerance tables are developed for comparing test results, then the data collected from non-mechanical seed counts may be used in labeling.

Note: should the proposal be adopted, the current sections for tolerances and reports of analysis will be renumbered, as well as Appendices 4 and 5.

Submitted by: Cindy Finneseth, Kentucky Ag. Experiment Station Seed Laboratory, (859) 257-5656**,** chfinn0@email.uky.edu and Deborah Meyer, California Department of Food and Agriculture, Plant Pest Diagnostics Center – Seed Science Laboratory, 916 262-1137, dmeyer@cdfa.ca.gov.

Date submitted: October 13, 2010.

2011 Rules Change Proposal 23

PURPOSE OF PROPOSAL: To fine-tune the current Uniform Blowing Procedure (UBP) for side-oats grama (*Bouteloua curtipendula*), keeping some of the efficiencies it offers, but hand-picking the light portion for florets containing caryopses at least 1/3 the length of the floret and classifying as pure seed. Also changing the PSU number 25 for seed lots comprised of only caryopses, to PSU number 21 for single florets and/or caryopses.

The seed industry has been indicating that the purity results on side-oats grama obtained with the UBP can be lower than their actual pure seed level. The third study in the supporting evidence showed an average loss of $2,211 per seed lot (table 6) when using the current UBP compared to the proposed method. Seed labs have been observing that various seed lots have a significant number of florets containing mature caryopses blowing over into the light portion during the UBP; primarily filled single florets. Some seed lots contain spikelet groups that break apart more readily than others. Seed lots in which the spikelet groups remain intact appear to give uniform and meaningful results using the current UBP. Currently, seed lots with spikelet groups that break apart, will result in lower purity results compared to their true seed quality. Some seed labs have been deviating from the AOSA rules by handpicking the units containing caryopses from the light portion, causing differences in test results among seed labs.

Present Rule:

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

Excerpt from Table 2A. Weights for working samples:

Pure Seed Unit #	Kind of Seed	Minimum weight for purity analysis[a] Grams	Minimum weight for noxious-weed seed or bulk examination Grams	Approximate number of seeds per gram[b] Number	Approximate number of seeds per ounce[c] Number
	Bouteloua curtipendula (Michx.) Torr.				
25	side-oats grama (caryopses)	2	20	1,605	45,640
23	(other than caryopses)	6	60	350	9,950

Excerpts from Table 3A. Pure seed unit definitions:

PSU Number	
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 Bouteloua curtipendula, in addition to the units described above, spikelet group that disarticulates as a unit with attached rachis and internode. *For *Bouteloua gracilis*, 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. *When coated seed units are de-coated for purity analysis, the Uniform Blowing Procedure shall not be used. A de-coated seed unit must contain at least one caryopsis with some degree of endosperm.

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

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 shall 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) 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) 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

(7) Side-oats grama: The equivalent air velocity value (m/s) for side-oats 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 1.480 to obtain the adjusted gate opening value for side-oats grama. The factor of 1.480 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 side-oats grama. Before blowing, remove any extraneous material that will interfere with the blowing process. 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:

5.5. Side-oats grama (*Bouteloua curtipendula*).

The equivalent air velocity value (m/s) for side-oats 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 1.480 to obtain the adjusted gate opening value for side-oats grama. The factor of 1.480 is restricted to the General-type Seed Blower; see sections 3.3 and 7.2 in the 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 side-oats grama.

The sample should be examined and large inert material removed that may interfere with the movement of seed in the blower. The sample should be divided into four approximately equal parts. Each part is blown separately with the large tube; the analyst should ensure that the seed units are moving freely in the blower. The four resulting heavy fractions are combined, and the same is done for the light fractions.

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

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. 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 for Testing Seeds 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 for Testing Seeds Vol. 1.

PROPOSED RULE:

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

Excerpt from table 2A. Weights for working samples:

Pure Seed Unit #	Kind of Seed	Minimum weight for purity analysis[a] Grams	Minimum weight for noxious-weed seed or bulk examination Grams	Approximate number of seeds per gram[b] Number	Approximate number of seeds per ounce[c] Number
21	*Bouteloua curtipendula* (Michx.) Torr. side-oats grama (single florets and/or caryopses)	2	20	~~1,605~~ 1,250	~~45,640~~ 35,437
23	(other than single florets and/or caryopses)	6	60	350	9,950
~~25~~	~~Side-oats grama (caryopses)~~	~~2~~	~~20~~	~~1,605~~	~~45,640~~

Excerpts from Table 3A. Pure seed unit definitions:

PSU Number	
21	Floret with attached empty floret(s) not extending to the tip of the fertile floret (excluding the awn), or single floret, provided there is a caryopsis at least one-third the length of the palea measured from the base of the rachilla. Caryopsis or piece of broken caryopsis larger than one-half of the original size. Special consideration: * A fertile floret attached to another fertile floret shall be separated. * Attached glumes and empty florets extending to or beyond the tip of the fertile floret shall be removed and classified as inert matter. * For Bouteloua curtipendula, this PSU only applies if the submitted sample consists primarily of single florets and caryopses. When the submitted sample consists primarily of

	multiple florets and spikelets refer to PSU 23.
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 Bouteloua curtipendula, in addition to the units described above, spikelet group that disarticulates as a unit with attached rachis and internode. In addition to the units described above, see section 3.6d(7) for the classification of the florets containing caryopses at least 1/3 the length of the floret found in the light portion. *For *Bouteloua gracilis*, 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. *When coated seed units are de-coated for purity analysis, the Uniform Blowing Procedure shall not be used. A de-coated seed unit must contain at least one caryopsis with some degree of endosperm.

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 shall 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 and 7) for blue grama and side-oats 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 and 7) for blue grama and side-oats 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

(7) Side-oats grama: The equivalent air velocity value (m/s) for side-oats 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 1.480 to obtain the adjusted gate opening value for side-oats grama. The factor of 1.480 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 side-oats grama. Before blowing, remove any extraneous material that will interfere with the blowing process. 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, weed seed, and inert matter are separated according to Sections 3.2-3.8. 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 inert matter, which was removed before the blowings, is added to the inert matter component of the sample. All remaining florets of side-oats 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. All side-oats grama seed units remaining in the heavy fraction are to be considered pure seed. However, caryopses that are broken and 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.

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

New last paragraph added to Introduction:
Gil Waibel added modifications to the Uniform Blowing Procedure (UBP) for side-oats grama in 2011, by changing the determination of pure seed and inert matter. This method continues the use of the UBP for side-oats 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. In addition, for seed lots comprised of single florets and/or caryopses the UBP does not apply (refer to PSU 21).

5.5. Side-oats grama (*Bouteloua curtipendula*).

The equivalent air velocity value (m/s) for side-oats 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 1.480 to obtain the adjusted gate opening value for side-oats grama. The factor of 1.480 is restricted to the General-type Seed Blower; see sections 3.3 and 7.2 in the 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 side-oats grama.

The sample ~~should~~ shall be examined ~~and large inert material~~ to remove~~d~~ extraneous material ~~that may interfere with the movement of seed in the blower~~ before blowing, and ~~The sample should be~~ then divided into four approximately equal parts. Each part is blown separately with the large tube; and the analyst ~~should~~ shall ensure by careful seed cup agitation that the seed units are moving freely in the blower. The four resulting heavy fractions are combined, and the same is done for the light fractions as instructed in section 6.7 of this volume.

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

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 inert matter, which was removed before the blowings, is added to the inert matter component of the sample. All remaining florets of side-oats 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 ~~O~~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. All side-oats grama seed units remaining in the heavy fraction are to be considered pure seed. However, caryopses that are broken ~~florets or~~ and ~~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.

HARMONIZATION AND IMPACT STATEMENT:

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

The two methods will be different, and 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 FSA 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 side-oats grama, and they are willing to suffer the consequences until, and if, the FSA is updated to be equivalent to this proposed AOSA method.

Canadian Methods and Procedures (M&P): The M&P does not list side-oats grama, so there is no conflict.

ISTA: There is currently a conflict with the ISTA rules. The ISTA pure seed definition for Bouteloua species (which includes side-oats grama) is “no need to check for the presence of a caryopsis.” ISTA does not use a blowing point for side-oats grama. The ISTA rule for side-oats 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 side-oats grama are produced and sold in the United States. There are a few seed lots sold in Mexico each year. Almost all side-oats grama is tested by the AOSA Rules and the Federal Seed Act, and very little side-oats grama is tested by the ISTA method.

SUPPORTING EVIDENCE:

There are three supporting studies attached to this proposal. The first is a comparison of purity working weights on side-oats single florets and caryopses, the second is a referee determining the uniformity of

the official and proposed methods on side-oats grama, and the third is a research study comparing the means of two methods (official and proposed methods) on side-oats grama.

AUTHOR'S NOTE: Due to the nature of the spikelet groups, and the chaffy nature of the species, achieving uniformity in testing side-oats grama is a challenge. Keeping the blowing point intact will give all labs a necessary (time-saving efficiency) and a uniform step before hand picking the light portion. If the UBP were discontinued for side-oats grama, seed analysts would be required to examine the spikelet groups to determine if there is a caryopsis in at least one of the florets in the spikelet group. The spikelet groups are fragile, and it is likely that the analyst could break some of the spikelet groups apart. Broken segments without any caryopsis would become inert matter, which would increase the inert percentage, and conversely the purity percentage would be altered. The differing techniques of each analyst would result in different amounts of broken spikelet groups, and this would make testing this species uniformly difficult. Keeping the UBP for side-oats grama eliminates the need for the analyst to examine each spikelet group. This modified UBP will improve the accuracy of this purity method, and in turn the current UBP has improved the uniformity over the use of the hand-picking method used years ago (the hand method alone did not achieve uniform results – see discussion of the history of testing side-oats grama described in the "background of the problem" in the third set of the supporting evidence to this proposal). Testing side-oats grama by hand without the UBP was very time-consuming, and the UBP greatly shortened the testing time. By keeping the UBP, there will be time savings with the mechanical separation, and by hand picking of the light portion for pure seeds, accuracy will be improved.

SUBMITTED BY:
Gil Waibel
Wyoming Seed Analysis Laboratory
749 Road 9
Powell, WY 82435-9135

PH: (307) 754-4750
FAX: (307) 754-4932
Email: gwaibel@uwyo.edu

DATE SUBMITTED: October 15, 2010 Revised January 20, 2011

Appendix 1 for Proposal 23

SUPPORTING EVIDENCE:
Three supporting studies follow:

1. Side-oats Grama Purity Working Weights of Single Florets and Caryopses
2. Referee: Determination of the Uniformity between Labs Using the Official and Proposed Uniform Blowing Procedure Methods on Side-oats Grama
3. Study: Mean Comparison of Purity Results between Two Uniform Blowing Procedures on Side-oats Grama

Supporting Evidence Study 1:
Side-oats Grama Purity Working Weights of Single Florets and Caryopses

The AOSA Rules currently require testing 2 grams of side-oats grama caryopses for a purity test, and 6 grams of (other than caryopses). This means single florets must be tested at the 6 gram amount. The following table compares the weight of 2500 single florets and caryopses.

Table 1: Comparison of Purity Working Weights between Side-Oats Grama Single Florets and Caryopses

		Single Floret			Caryopses	
Lot #	Variety	100 seeds Grams	2500 seeds Grams		100 seeds Grams	2500 seeds Grams
1	Native	0.0947	2.3675		0.0716	1.7900
2	El Reno	0.0833	2.0825		0.0747	1.8675
3	Pierre	0.1001	2.5025		0.0803	2.0075
4	Haskell	0.0863	2.1575		0.0586	1.4650
5	Niner	0.0769	1.9225		0.0486	1.2150
6	Vaughn	0.1016	2.5400		0.0793	1.9825
7	Butte	0.0857	2.1425		0.0653	1.6325
8	Vaughn	0.0901	2.2525		0.0710	1.7750
Average			2.2459			1.7169

The average weights for both the side-oats grama single florets and caryopses (table 1), round to 2.0 grams (2.2459+1.7169=3.9628; 3.9628/2=1.9814 which rounds to 2.0 grams). Single units of side-oats grama are marketed as a mix of single florets and caryopses, and should this proposal pass, the correct PSU number in table 3A of the AOSA Rules would be #21, not #25. In table 2A of the AOSA Rules, it would be necessary to change the approximate number of seeds per gram from 1,605 to 1,250, and to change the approximate number of seeds per ounce from 45,640 to 35,437. When the caryopses and the single floret weights (2,500 seeds) of the eight seed lots in this study are averaged (2.2459+1.7169=3.9628; 3.9628/2=1.9814) and divided by 2500 seeds, the result is .0008 grams per seed unit. Dividing the weight per seed unit into 1 gram results (1/0.0008) in 1,250 seeds per gram, and multiplying this result by 28.3494 grams/ounce results in 35,437 seeds per ounce.

Supporting Evidence Study 2:
Referee:
Title: Determination of the Uniformity between Labs Using the Official and Proposed Uniform Blowing Procedure (UBP) Methods on Side-oats Grama

Purpose: To compare the results of 7 seed laboratories testing 6 seed lot samples to see if a modified UBP of side-oats grama improves the uniformity and accuracy of results compared to the official UBP method.

Materials and Methods:
Participating labs were, Arkansas Valley Seed Solutions Lab, Iowa State University Seed Lab, North Dakota State Seed Laboratory, South Dakota State University Seed Laboratory, Texas Department of Agriculture – Stephenville Seed Laboratory, Nebraska Crop Improvement Association Seed Laboratory, and the Wyoming Seed Analysis Laboratory. Six samples of differing purity levels were used. The samples were divided at the Wyoming Seed Analysis Lab into replicates of approximately 1.5 grams each, to assure uniformity in the dividing procedure. Each replicate was ¼ of an official purity sample for side-oats grama. Each lab tested four 1.5 gram replicates of each sample.
Other materials required were the general seed blower, anemometer and scales capable of weighing seeds to a ten thousandth of a gram (0.0001 gram) were used in the blowing procedures and purity analysis. Each laboratory has had their seed blower calibrated with the master Kentucky bluegrass sample. In determining if florets blown over into the light portion contained caryopses at least 1/3 the length of the floret, each lab used their own standard equipment and methodology.

Purity Procedure:
All extraneous material was removed from the sample before each blowing.
Blower setting was checked before each blowing with an anemometer.
Each replicate was placed in the 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.
The 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 when the proposed purity method was calculated, and they remained as part of the inert portion when the official method was calculated. All other crop and weed seeds were removed as appropriate.
All seed units in the heavy portion were considered pure seed. All other crop, weed seed, and inert matter (other than empty florets) were removed as appropriate.
Purity results were returned to the Wyoming Seed Analysis Lab for percentage calculation, compilation and analysis. The data was collected in a way that the official and proposed methods could be calculated and compared.
Statistical analysis:
The data collected were analyzed using a randomized complete block design with a (2 X 7 X 6) factorial arrangement. All samples were replicated 4 times.
2=factor A (official and proposed methods)
7=factor B (laboratories)
6=factor C (seed lots)
All means were separated using Duncan's multiple range test at 0.05 probability level.

Results and Discussion:

Table 1: Analysis of Variance (Factor A=2 methods, Factor B=7 laboratories and Factor C=6 seed lots)

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Value	Prob
Replication	3	46.433	15.478	3.6145	0.0139
Factor A	1	3520.199	3520.199	822.0760	0.0000
Factor B	6	5103.703	850.617	198.6456	0.0000
AB	6	1113.573	185.595	43.3423	0.0000
Factor C	5	25336.752	5067.350	1183.3841	0.0000
AC	5	2758.480	551.696	128.8382	0.0000
BC	30	2287.989	76.266	17.8106	0.0000
ABC	30	924.279	30.809	7.1949	0.0000
Error	249	1066.239	4.282		
Total	335	42157.645			

Coefficient of Variation 2.62%

There were significant differences between seed lots, methods, and labs, as well as their interactions (table 1). There were also significant differences between replicates, which was not unexpected due to the natural uniformity issues within side-oats grama.

Table 2: Pure Seed Mean Comparison using Official and Proposed UBP Methods by 7 Seed Laboratories on 6 Seed Lots

Lab Number	Six Lot Pure Seed Mean Using the Official Method %	Significance Rank	Six Lot Pure Seed Mean Using the Proposed Method%	Significance Rank
1	75.42	F	83.07	BC
2	64.23	H	76.44	F
3	81.63	D	84.71	A
4	79.45	E	80.11	E
5	80.21	E	84.71	A
6	74.05	G	82.81	CD
7	75.69	F	84.16	AB
Overall Average	75.81		82.29	

Duncan's Multiple Range Test (LSD=1.177). Means followed by the same letter are not significantly different while those followed by different letters are significantly different at 5% probability.

There was a significant difference between the overall average of the official and proposed UBP methods (table 2). The overall average of the proposed method (82.29%) was higher than that with the official method (75.81%). The referee resulted in two out of seven seed labs with a significance rank of E and two labs with a significance rank of F (each significance rank was not significantly different) using the official UBP method. None of the other labs results using the official method agreed significantly. Using the proposed method, there were three out of seven seed labs with a significance rank of A, two labs with a significance rank of B, and two labs with a significance rank of C. This indicates more uniform results will be gained using the proposed method.

Conclusion:

The results of the official AOSA UBP for side-oats grama showed significant differences between all but 4 labs (table 2). The proposed method gave more uniformity between the significance ranks of the 7 labs.

The main difference between labs appears to be either the calibration or performance of the seed blower. All labs have used the master AOSA calibration sample, and these testing differences were unexpected. The official UBP simply used the blowings to separate the material, and this should have given more uniform results between labs. There was also a difference between the labs in determining which seeds in the light portion of the blowings contained caryopses at least 1/3 the length of the floret. The AOSA rules are not specific on how to determine the presence of a caryopsis in a floret of any grass species. Some labs use microscopic magnification while applying slight pressure on the floret, while other labs utilize a diaphanoscope while using slight pressure with differing levels of magnification. The method of determining the presence of a caryopsis in a floret in this referee was purposely left undefined, because the purpose of the referee is to see what kind of uniformity is gained in the "real world" of seed testing.

Using the AOSA tolerances from table 13A, each OM and PM average percentage by each seed lab was subtracted from the high OM and PM average percentage respectively, and the following table was compiled (table 3).

Table 3: Determination of Tolerance Using AOSA Table 13A Comparing Seven Lab Pure Seed Averages on Two Methods on Six Seed Lots

Lab Number	Official Method (OM) Pure Seed Mean on 6 Lots (%)	OM Difference from high result (%)	OM In or Out of Tolerance Using AOSA Table 13A	Proposed Method (PM) Pure Seed Mean on 6 Lots (%)	PM Difference from high result (%)	PM In or Out of Tolerance Using AOSA Table 13A
1	75.42	6.21	Out(Tol=3.18)	83.07	1.64	In (Tol=2.76)
2	64.23	17.40	Out (3.55)	76.44	8.27	Out (3.09)
3	81.63	0.00	In (2.88)	84.71	0.00	In (2.62)
4	79.45	2.18	In(2.99)	80.11	4.60	Out (2.88)
5	80.21	1.42	In (2.88)	84.71	0.00	In (2.62)
6	74.05	7.58	Out (3.18)	82.81	1.90	In (2.76)
7	75.69	5.94	Out (3.18)	84.16	0.55	In (2.62)
Overall Average	75.81	5.82	Out (3.18)	82.29	2.42	In (2.76)

The results of the proposed modified UBP show less differences than the official UBP (table 3). The range from high to low for the official UBP method is 17.40 percent compared to 8.27 percent for the proposed method. If lab 2 is dropped, the range between the high and low is 7.58 percent for the official method compared to 4.60 percent for the proposed method. The official UBP had only 3 out of 7 seed labs in tolerance with the high OM mean. The proposed modified UBP had 5 out of 7 seed labs in tolerance with the high PM mean. Comparing the results to the low lab is not practical because it was considerably lower than all other labs. The overall average of the official method is not in tolerance, and the overall average of the proposed method is in tolerance, both with the high mean in their respective columns. These data show that the proposed method achieves more uniform results between seed labs. By removing seeds with caryopses at least 1/3 the length of the floret from the light portion, and adding these seeds to the pure seed component of the purity achieves a more accurate estimate of the seed lots pure seed percentage.

Supporting Evidence Study 3:
Mean Comparison of Purity Results between Two Uniform Blowing Procedures (UBP) on Side-oats Grama
(*Bouteloua curtipendula*)

Background of the problem:
The original official purity technique in the AOSA Rules for Testing Seeds[1] to determine pure seed in side-oats grama was to use the hand method. According to A.L. Larson and L. E. Wiesner this was a very time consuming method, and results were not consistent between labs.[2,3] There have been questions by seed analysts and seed industry members since the UBP of side-oats grama became part of the AOSA rules in 1982, about the accuracy of the UBP. "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 side-oats grama.[3,4] 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.[3] Wiesner stated that the modified method was designed to make the purity results more consistent .[3] Larry Prentice, chair of the AOSA Rangegrass Research Subcommittee, in a 1982 rule proposal, mandated using the UBP for side-oats grama.[5] The Rangegrass Committee published a referee comparing three different purity methods including the UBP for side-oats grama, and also recommended using the UBP.[6] The UBP for side-oats grama became the official method for the AOSA rules in 1982.[7] The UBP brought more consistency in purity results, but some accuracy was lost. Members of the seed industry have claimed that they have been losing millions of dollars since the use of the UBP, and would like a more accurate method developed. Industry members are willing to pay the additional costs needed to test side-oats grama, because of lost revenue due to the mechanically arbitrary way the UBP can send "pure seeds" into the light portion in differing amounts from seed lot to seed lot.

Rational:
Time savings and backlogs are important, and timely results are critical during certain seasons of the year. However, the AOSA rules also challenges us that "in all cases the ultimate purpose of making a test is to determine the value of the seed for planting."[1] This study will determine if the official UBP was both consistent and accurate compared to hand-picking florets with caryopses in the light portion of the UBP. The purity percentage was determined on thirteen seed lots using the following methods:

1. Official method using the blowing point (Official UBP Method "OM")
2. Hand-picking florets with a caryopses at least 1/3 the length of the floret from the light portion of the UBP, and including the weight of these florets with the pure seed. (Proposed UBP Method "PM")

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

1. If "pure seeds" are blowing over into the light portion;
2. If there are significant differences in purity results between the proposed UBP method (PM) and the official UBP method (OM);
3. If there is a way to fine-tune the UBP to be more accurate.

Seed sources:
Samples from thirteen seed lots were selected from various sources: varieties (7 varieties which includes one native collected), areas of production (6 states), years of production (2 seed lots from 2006, 4 from 2007, 4 from 2008, and 3 from 2009), irrigated (10 yes, 3 no), and certified (5 yes, and 8 no) (table 1).

Table 1: Seed Source

Sample	Variety	Origin	Year Produced	Irrigated	Certified
1	Native (Collected)	Oklahoma	2007	No	No
2	Butte	Kansas	2006	Yes	No
3	Pierre	South Dakota	2008	No	No
4	Haskell	Texas	2007	Yes	No
5	Vaughn	New Mexico	2006	Yes	Yes
6	El Reno	Texas	2008	Yes	Yes
7	Vaughn	Texas	2008	Yes	No
8	Niner	Colorado	2008	Yes	Yes
9	El Reno	Texas	2007	Yes	No
10	Niner	Colorado	2007	Yes	Yes
11	Vaughn	Texas	2009	Yes	No
12	El Reno	Colorado	2009	No	No
13	Niner	Colorado	2009	Yes	Yes

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 1.5000+ grams were individually divided using the Gamet Seed Divider
2. Stems were removed before using blowing procedure
3. Blower was checked with anemometer before each blowing (setting based on calibration obtained with the master calibration sample)
4. Replicate was blown at the official setting for side-oats grama (using the Kentucky bluegrass blowing point which was multiplied by a factor of 1.480)
5. Remaining inert (not empty florets) and other species if any were removed from the heavy portion and the “official” purity method 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 considered inert matter for the official method; and 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 and thus defined as pure seed) for the proposed method.

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

1. Official UBP method (OM).
2. Proposed modified UBP method (PM) – Use official UBP for side-oats grama, and follow up by hand-picking seed units with caryopses at least 1/3 the length of the floret from light portion, and add to the pure seed component of the purity.

Experimental design: The data collected were analyzed using a randomized complete block design with a (2 X 13) factorial arrangement. All samples were replicated 4 times.
2=factor A (official and proposed methods)
13=factor B (seed lots)

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 seed lots and methods of separating pure seeds from side-oats grama had significant effect on the percentage of pure seed results (table 2).

The pure seeds separated from the thirteen samples ranged between 50.81% and 95.00% using the official UBP method (OM) (table 3). This reflects the effect of seed production practices, origin of seed lot, weather conditions, variety, time of harvest, and level of seed cleaning on the final purity of a sample.

Table 2: Analysis of Variance of Side-oats Grama Purity Results Using 2 Methods and 13 Seed Lots

K		Degrees of	Sum of	Mean	F	
Value	Source	Freedom	Squares	Squares	Value	Prob
1	Rep	3	75.925	25.308	3.5236	0.019
2	Lot	12	10112.916	842.743	117.3344	0.0000
4	Method	1	208.345	208.345	29.0077	0.0000
6	Interaction	12	383.970	31.998	4.4550	0.0000
7	Error	75	538.680	7.182		
	Total	103	11319.836			

Coefficient of Variation: 3.25%

There were significant differences between seed lots, and methods (table 2). There were also significant differences between replicates, which was not unexpected due to the natural uniformity issues within side-oats grama.

Table 3: Mean Purity Comparisons between the Official UBP Method (OM) and the Proposed Modified UBP Method (PM) for 13 Seed Lots of Side-oats Grama

Seed Lot#	Official Method Purity %	Significance Rank	Proposed Method Purity %	Significance Rank
1	95.00	A	95.03	A
2	91.81	AB	92.18	AB
3	93.46	A	93.61	A
4	80.40	FG	80.75	FG
5	82.62	F	84.15	DEF
6	74.20	I	78.18	GH
7	72.64	I	73.04	I
8	86.92	BC	87.37	BC
9	50.81	K	63.25	J
10	88.60	BC	94.35	A
11	81.29	FG	82.89	EF
12	82.14	FG	82.57	F
13	75.19	I	84.50	CDEF
Average	81.16		83.99	

Duncan's Multiple Range Test (LSD=3.775). Means followed by the same letter are not significantly different while those followed by different letters are significantly different at 5% probability.

There was a significant difference between the official and proposed methods (table 3). The percent seed purity by the proposed method (83.99%) was higher than that with the official method (81.16%). Of the 13 seed lots tested, lots 6, 9, 10 and 13 had significant differences between the official and the proposed UBP methods.

Table 4: Four Replicate Total of Side-oats Grama Seed Units Removed from the Light Portion of the UBP

Seed Lot#	Light Portion			
	# Pure Florets Found in Light Portion	Weight of Pure Seeds Found in the Light Portion (grams)	Weight Tested (grams)	Percent Pure Florets in Light Portion
1	1	0.0017	6.2293	0.03
2	13	0.0226	6.1475	0.37
3	7	0.0093	6.0712	0.15
4	18	0.0214	6.1018	0.35
5	57	0.0920	6.0172	1.53
6	222	0.2428	6.0966	3.98
7	29	0.0245	6.1131	0.40
8	17	0.0051	6.1277	0.08
9	691	0.7609	6.1181	12.44
10	459	0.3492	6.0623	5.76
11	68	0.0997	6.2339	1.60
12	22	0.0277	6.3800	0.43
13	581	0.5948	6.3888	9.31
Average	168	0.1732	6.1606	2.81

Every sample had florets with caryopses in the light portion ranging from 1 to 691 florets (table 4). An average of 168 pure seeds was pulled out of the light portion of the blowings. All 13 seed lots averaged an increase of 2.81% when comparing the proposed modified UBP method to the official UBP method.

Table 5: Pure Seed and Total Viability Percentages of Side-oats Grama on Thirteen Seed Lots, Based on the Official (OM) and the Proposed (PM) UBP methods

Seed Lot#	Pure Seed Percent Official Method (OM)	Pure Seed Percent Proposed Method (PM)	Total Viable Official Method (OM) %	Total Viable Proposed Method (PM) %
1	95.00	95.03	67	68
2	91.81	92.18	74	74
3	93.46	93.61	79	78
4	80.40	80.75	54	59
5	82.62	84.15	71	67
6	74.20	78.18	88	79
7	72.64	73.04	45	51
8	86.92	87.37	80	81
9	50.81	63.25	89	86
10	88.60	94.35	98	99
11	81.29	82.89	68	72
12	82.14	82.56	27	26
13	75.19	84.50	86	84
Mean	81.16	83.99	71	71

Table 5 is included to help calculate the pure live seed (PLS) percentage in table 6 to illustrate the financial impact the official method has on the seed industry by blowing florets with caryopses into the light portion. The total viable percentages are from 400 seed germination tests on each seed lot, and from each purity method. Obtaining the same average total viable result on each method (71 percent) on all 13 seed lots was a surprise. In general, one would think that the proposed method would result in a slightly lower average viability percentage, since seeds from the light portion added to the pure seed in the heavy portion would have a slightly lower viability than the seeds in the heavy portion. However, we are dealing with spikelet groups in the heavy portion, and in some cases, and in differing amounts between seed lots, there will be seed units in the heavy portion that have no caryopses at all. Adding a significant number of pure seeds from the light portion did cause the viability percentage to increase on some seed lots when using the modified UBP.

Table 6: Financial Impact (Based on Thirteen Seed Lots of Side-oats grama) on the Seed Industry between Two Purity Methods

	Official Method (OM)	Proposed Method (PM)
Pure Seed % Mean of 13 lots	81.16	83.99
Total Viability Mean of 13 lots	71	71
PLS=(Purity X Total Viable)/100	57.62	59.63
PLS on 20,000# lots	11,524	11,926
$/PLS	5.50	5.50
Total $ value of lot	$63,382	$65,593
$ loss compared to OM		$-2,211

Table 6 shows that when comparing the official UBP method (OM) to the proposed UBP method (PM), the average decreased value on a 20,000 lb. (average size) seed lot is $2,211.

Conclusion:

Answers to the objectives stated for this study follow:

1. ***If "pure seeds" are blowing over into the light portion.*** All 13 samples had florets with caryopses blown to the light portion when the official method (OM) was applied. A range of 1 to 691 florets with caryopses blew into the light portion of the UBP (table 4). An average of 168 florets with caryopses blew into the light portion of the UBP per seed lot.
2. ***If there are significant differences between the proposed UBP method (PM) and the official UBP method (OM).*** Four of the thirteen samples tested were significantly different using Duncan's Multiple Range Test (table 3). Averaging the florets with caryopses in the light portion on all thirteen seed lots, resulted in a 2.81% gain in pure seed for the proposed method (table 4).
3. ***If there is a way to fine-tune the UBP to be more accurate.*** The results of this study indicate that UBP can be "fine-tuned" by hand-picking the light portion for florets with caryopses, and classifying these florets as pure seed.

Based on the above studies, fine-tuning the official UBP as proposed will produce more accurate, consistent results, while maintaining some of the efficiency gained with the mechanical seed blower. It is the seed grower who can be hurt the most, since once they sell, they are out of the market. Seed companies buy and sell seed based on the test results defined by the AOSA rules so they can expect consistency from test to test, and the pure seed value of their seed lots should not change. Seed companies gain by having satisfied and high quality seed growers. If a seed grower thinks they should have a higher purity percentage, and finds that a significant number of "pure seeds" are blowing over during the UBP, they will be understandable disappointed. Many dollars, as well as quality seed growers, have been lost by the side-oats grama seed industry, and saving a few seed testing dollars by using the official method, is far out-weighed by the losses passed on to seed growers. The seed industry has been asking for a solution to this problem since we began using the UBP in 1982, and this study has reinforced their concern.

References:

1. AOSA Rules for Testing Seeds, Vol. 1 Principles and Procedures. 2010 edition: 3-7 – 3-9.
2. Larsen, A.L. 1977. Special problems in rangegrass analysis. AOSA News Letter 51(1):42
3. Wiesner, L.E. 1977. Rules Committee. Proposals for rules changes. AOSA News Letter 51(2):8
4. Wiesner, L.E. 1977. Rules Committee. AOSA News Letter 51(4):37
5. Prentice, L. 1982. Rules Committee. Proposed rule changes. AOSA News Letter 56(1):38-39
6. Research Subcommittee on Rangegrass Analysis. 1982. Comparison of Three Methods of Testing Seeds of Side-oats Grama 1981. AOSA News Letter 56(1):51-59
7. AOSA Business Meeting. 1982. Minutes. AOSA News Letter 56(3):14-17

2011 Rules Change Proposal 25 Amended

Purpose: Change the title of Table 13H and further clarify the use of the tolerances for comparing the percentage of pure seed of two tests based on the fluorescence test of ryegrass, the copper sulfate-ammonia test for sweet clover or other tests for separation of species based on morphological characteristics.

Present Rule:

13.4 Tolerances for fluorescence test of *Lolium* and 400 — 1000-seed separations in purity analysis.

These tolerances are based on the requirement that when two independent trials or tests have been made on the same properly mixed bulk lot, the chances of divergence between the two trials exceeding the tolerance will not be greater than one in twenty. (For the formula see Leggatt, 1939).

The tolerances in Table 13H recognize the sampling error introduced by the small number of seeds used and are to be applied in determining the variations for those factors affected by the use of a small number of seeds. If results of 400 to 1000-seed separations or fluorescence tests of ryegrass are used in computing the percentages of pure seed, one-half the regular pure seed tolerance shall be added to the tolerance for the 400 to 1000-seed separation. The tolerances to be used have been computed to a certainty of five percent (P = 0.05) and are given in Table 13H. These tolerances are to be determined from the result or results found by test.

Method of using Table 13H: Enter the percentage to which the tolerance is to be applied in the left-hand column. Next, find in the top horizontal row the number of seeds used in your test and to which the tolerance is to be applied; then, find in the second row the number of seeds used in the test with which your results are to be compared. (If the number of seeds is not known, assume that 400 seeds were used.) The corresponding tolerance will be found in the column below. This figure plus one-half the pure seed tolerance should be added to the percentage for which tolerance is required. Tolerances for fractions of percentages entered in column 1 should be interpolated. Thus, the tolerance in the 400/400 seed column for 94.5 percent is 2.75 (Table 13H).

Examples:

Fluorescence test of ryegrass. Refer to sec. 5.2b(2).

For one-way comparison of second test against the first test (label).

Seed lot label states:	Perennial ryegrass	92.5 %
	Other crop	4.00%
	Inert matter	3.36%
	Weed seed	0.14%

Test Results *(second test):*

When a test is conducted to determine if the label is correct 98.49% *Lolium* spp. is found. A 400-seed fluorescence test is conducted to determine the percentage of perennial ryegrass. The fluorescence level of variety being tested = 0%.

#

360 Non-fluorescent seedlings = 90%
40 Fluorescent seedlings = 10% test fluorescence (TFl)
400 total seedlings tested

Calculation of tolerance:

$\frac{360}{400}$ x 100 = 90.00% pure seed (perennial ryegrass) by number

Tolerance for Test Fluorescence test result of 90% ($\frac{400}{400}$) column = 3.8%

+ $\frac{1}{2}$ pure seed tolerance (Table 13B column D) for 98.40% = 0.47%

Total Tolerance = 4.27%

Application of tolerance:

Determine the percentage of perennial ryegrass in the sample [refer to sec. 5.2b(2)].

$\frac{100\%-10\%}{100\%-0\%}$ x 98.40% = 88.56% perennial ryegrass in sample

Difference of the two test results: 92.50 – 88.56 = 3.94%

Since the difference in the percentage of perennial ryegrass between the two tests (3.94%) does not exceed the tolerance (4.27%), it is not considered significant (i.e., due to random sampling variation); therefore, the label is satisfactory as far as the percentage of pure seed (perennial ryegrass) is concerned.

Chemical test for sweetclover (Refer to section 5.2a(3).

Use for a one-way comparison of second tests against the first test or label.

Seed lot label states:	White sweetclover	98.55%
	Other crop	0.00%
	Inert matter	1.25%
	Weed seed	0.20%

Test Results *(second test):*

When a purity test is conducted to determine if the label is correct, 98.76% pure *Melilotus* spp. seed was found. A cupric-sulfate test was conducted using 400 seed.

368 stained olive or yellow-green; identified as whille sweetclover; therefore classified as pure seed
32 stained dark brown or black; identified as yellow sweetclover; therefore classified as other crop seed
400 total seed tested

Calculation of tolerance:

#

$\frac{368}{400}$ x 100 = 92.00% pure seed by number

Tolerance for 92% olive or yellow-green seeds ($\frac{400}{400}$) column = 3.4%

+ $\frac{1}{2}$ pure seed tolerance (Table 13B column C) for 98.76% = 0.33%

Total Tolerance = 3.73%

Application of tolerance:

Determine the percentage of white sweetclover in the sample [refer to sec. 5.2a(3)].

% of white sweetclover in sample = $\frac{92.00 \, x \, 98.76}{100}$ = 90.86%

Difference of the two test results: 98.55 – 90.86 = 7.69%

Since the difference in the percentage of white sweetclover between the two tests (7.69%) exceeds the tolerance (3.73%), it is considered significant (i.e., the two results are not within tolerance).

Separation of species based on morphological characters (e.g., species of Agrostis, *Brassica, melilotus, Poa, and cultivar determination of Avena, Sorghum,* etc.). Refer to sec. 3.1c.

Use for one-way comparison of second test against the first test or label.

Seed lot label status:	Kentucky bluegrass	92.50%
	Other crop	4.00%
	Inert matter	3.36%
	Weed seed	0.14%

Test Results *(second test):*

When a test is conducted to determine if the label is correct, there appear to be two species of *Poa* in the sample. The *Poa* spp. comprises 95.43% of the sample. A 1000-seed separation is conducted to determine the percentages of Kentucky bluegrass and other species.

928	Kentucky bluegrass	0.3027 g = 91.31% by weight of 1000 seed units.
72	Canada bluegrass	0.0288 g = 8.69% by weight of 1000 seed units
1000		0.3315 g

Calculation of tolerance:

$\frac{928}{1000}$ x 100 = 92.8%

Tolerance for 92.8% for 1000 seeds ($\frac{1000^a}{400}$) column = 2.84% (interpolated)

+ $\frac{1}{2}$ pure seed tolerance (Table 13B column D) for 95.43% = 1.51%

#

Total Tolerance = 4.35%

Application of tolerance:

Determine the percentage of Kentucky bluegrass in the sample (refer to sec. 3.1c).

$$\% \text{ of Kentucky bluegrass in sample} = \frac{91.31 x\ 95.43}{100} = 87.14\%$$

Difference test results: 92.50 – 87.14 = 5.36%

Since the difference in the percentage of Kentucky bluegrass between the two tests (5.36%) exceeds the tolerance (4.35%), it is considered significant (i.e., the two results are not within tolerance).

[a] The figure 400 is used since these rules provide that at least 400 seeds shall be used in tests of this kind. If the number of seeds is not known, assume that 400 seeds were used.

Table 13H. Tolerances for 400, 800, and 1000-seed tests for comparing the percentage of pure seed from two independent tests from the same seed lot analyzed in the same or in different laboratories (*P*=0.05)

No. seeds in test to which Tolerance is to be applied	400	400	800	800	400	800	1000	1000
No. of seeds used in other test	400	800	400	800	1000	1000	1000	800

No change in the tolerance values of Table 13H, only the title and the left column headings will be changed.

Proposed Rule:

New changes inserted in blue, and omitted text is strikethrough with black.

13.4 Tolerances for comparing the percentage of pure seed of two tests based on the fluorescence test for ryegrass, the copper sulfate-ammonia test for sweet clover or other tests used for the separation of species based on morphological characteristics.

These tolerances are ~~based on the requirement that~~ applicable when two independent ~~trials or~~ tests have been made on the same properly mixed bulk lot. The chances of ~~divergence~~ differences between the two ~~trials~~ tests exceeding the tolerance will not be greater than ~~one in twenty~~ 5%. (~~For the formula see~~ Leggatt, 1939).

The tolerances in Table 13H recognize the sampling error introduced by the small number of seeds used and are to be applied in determining the variations for those factors affected by the use of a small number of seeds. If ~~results of 400 to 1000 seed separations or~~ the fluorescence tests of ryegrass, the copper sulfate-Ammonia test for sweet clover, or any test uses morphological characteristics to separate species are used in computing the percentages of pure seed, one-half of

the regular pure seed tolerance shall be added to the tolerance for the 400 to 1000-seed separation. The tolerances to be used have been computed to a certainty level of five percent (P = 0.05) and are given in Table 13H. ~~These tolerances are to be determined from the result or results found by test.~~

Method of using Table 13H: Enter the percentage of pure seeds of the second test that is based on the fluorescence test for ryegrass, the copper sulfate test for sweet clover, or any other morphological test to which the tolerance is to be applied in the first left-hand column. Next, find in the very top horizontal row the number of seeds used in your test (i.e., the second test that is conducted to verify a label or a first test) and to which the tolerance is to be applied; then, find in the second horizontal row the number of seeds used in the first test (or label) with which your second test results are to be compared. (If the number of seeds is not known, assume that 400 seeds were used.) The corresponding tolerance will be found in the column below. This tolerance figure plus one-half the pure seed tolerance (from the second test before the fluorescence test or any other test is conducted) should be ~~added to the percentage for which~~ the final tolerance value to be considered. Tolerances for fractions of percentages entered in column 1 should be interpolated. Thus, the tolerance in the 400/400 seed column for 94.5 percent is 2.75 (Table 13H).

Example of interpolation:

What is the interpolated tolerance value of the purity percentage of 94.5?

(Refer to Table 13H)

- According to Table 13H, the interpolated tolerance value of 94.5% pure seed of 400/400 tests lies between 2.6 and 2.9

- Since 94.5% purity value lies between 95% and 94%; look first for the tolerance values of 95% purity (1st column) under 400/400 seed tests (2nd column), it is 2.6. Then look for the tolerance values of 94% purity under the same column, it is 2.9

The calculation of the interpolated tolerance value for 94.5% purity is as follows:

$$X= 2.6 + [(2.9 – 2.6) \times (95-94.5)] = 2.75$$

It can also be identified as:

$$X= 2.9 – [(2.9-2.6) \times (95 – 94.5)] = 2.75$$

The interpolation formula is:

$$X = x_0 + [(x_1 – x_0)\ x\ (y – y_0)]; \quad \text{or} \quad X = x_1 - [(x_1 – x_0)\ x\ (y – y_0)]$$

Where, x is the interpolated tolerance value need to be identified, x_0 is the lower tolerance value (in the range of purity percentage), x_1 is the higher tolerance value (in the range of purity percentage), y is the higher purity percentage, y_0 is the specific purity percentage where the interpolated tolerance value need to be identified.

Examples:

Fluorescence test of ryegrass. Refer to sec. 5.2b(2).

For one-way comparison of a second test against a first test (or a label).

Seed lot label states:	Perennial ryegrass	92.5 %
	Other crop	4.00%
	Inert matter	3.36%
	Weed seed	0.14%

Test Results *(second test):*

When a test is conducted to determine if the label is correct 98.40% *Lolium* spp. is found. A 400-seed fluorescence test is conducted to determine the percentage of perennial ryegrass. The varietal fluorescence level (VFL) of variety being tested = 0%.

Determination of pure perennial ryegrass in the second test using the TFl [Refer to section 5.2b (2)].

360 Non-fluorescent seedlings = 90%
40 Fluorescent seedlings = 10% test fluorescence (TFl)

400 Total seedlings tested

Perennial ryegrass in sample (by weight) = $\frac{100\%-10\%}{100\%-0\%}$ x 98.40% = 88.56%

Difference between the first test (or label) and the second test results: 92.50 – 88.56 = 3.94%

Calculation of tolerance (Refer to Table 13H):

Pure perennial ryegrass seed (by number) using the TFl = $\frac{360}{400}$ x 100 = 90.00%

Tolerance for perennial seed of 90% according to the TFl for ($\frac{400}{400}$) column = 3.8%

+ $\frac{1}{2}$ pure seed tolerance (Table 13B column D) for 98.40% = 0.47%

Total Tolerance = 4.27%

Application of tolerance:

Since the difference in the percentage of perennial ryegrass between the two tests (3.94%) does not exceed the tolerance (4.27%), it is not considered significant (i.e., due to random sampling variation); therefore, the label is satisfactory as far as the percentage of pure seed (perennial ryegrass) is concerned.

Copper sulfate-ammonia test for sweet clover [Refer to section 5.2a(3)].

Use for a one-way comparison of second tests against the first test or label.

Seed lot label states:	White sweetclover	98.55%
	Other crop	0.00%
	Inert matter	1.25%

#

	Weed seed	0.20%

Test Results *(second test):*

When a purity test is conducted to determine if the label is correct, 98.76% pure *Melilotus* spp. seed was found. A cupric-sulfate test was conducted using 400 seed.

368 Stained olive or yellow-green; identified as while sweet clover; therefore classified as pure seed
32 Stained dark brown or black; identified as yellow sweet clover; therefore classified as other crop seed

400 Total seed tested

Determine the percentage of white sweet clover in the sample [refer to sec. 5.2a(3)].

Pure white sweet clover in the sample (by weight) = $\frac{92.00 \, x \, 98.76}{100}$ = 90.86%

Difference between the first test (or label) and the second test results: 98.55 – 90.86 = 7.69%

Calculation of tolerance:

Pure seed (by number) = $\frac{368}{400}$ x 100 = 92.00%

Tolerance for 92% olive or yellow-green seeds ($\frac{400}{400}$) column = 3.4%

+ $\frac{1}{2}$ pure seed tolerance (Table 13B column C) for 98.76% = 0.33%

Total Tolerance = 3.73%

Application of tolerance:

Since the difference in the percentage of white sweet clover between the two tests (7.69%) exceeds the tolerance (3.73%), it is considered significant (i.e., the two results are not within tolerance).

Separation of species based on morphological characters (e.g., species of Agrostis, *Brassica, melilotus, Poa, and cultivar determination of Avena, Sorghum*, etc.). Refer to sec. 3.1c.

Use for one-way comparison of second test against the first test or label.

Seed lot label status:	Kentucky bluegrass	92.50%
	Other crop	4.00%
	Inert matter	3.36%
	Weed seed	0.14%

Test Results *(second test):*

When a test is conducted to determine if the label is correct, there appear to be two species of *Poa* in the sample. The *Poa* spp. comprises 95.43% of the sample. A 1000-seed separation is conducted to determine the percentages of Kentucky bluegrass and other species.

928 Kentucky bluegrass 0.3027 g = 91.31% by weight of 1000 seed units.

#

72 Canada bluegrass 0.0288 g = 8.69% by weight of 1000 seed units
1000 0.3315 g

Determine the percentage of Kentucky bluegrass in the sample (Refer to sec. 3.1c).

Pure Kentucky bluegrass in sample (by weight) $= \frac{91.31 x\ 95.43}{100} = 87.14\%$

Difference between the first test (or label) and the second test results: 92.50 – 87.14 = 5.36%

Calculation of tolerance:

Pure seed (by number) = $\frac{928}{1000}$ x 100 = 92.8%

Tolerance for 92.8% for 1000 seeds ($\frac{1000^a}{400}$) column = 2.84% (interpolated)

+ $\frac{1}{2}$ pure seed tolerance (Table 13B column D) for 95.43% = 1.51%

Total Tolerance = 4.35%

Application of tolerance:

Since the difference in the percentage of Kentucky bluegrass between the two tests (5.36%) exceeds the tolerance (4.35%), it is considered significant (i.e., the two results are not within tolerance).

[a] The figure 400 is used since these rules provide that at least 400 seeds shall be used in tests of this kind. If the number of seeds is not known, assume that 400 seeds were used.

New title for Table 13H.

Table 13H. Tolerances for comparing the percentage of pure seed of two independent tests of 400, 800 or 1000 seeds from the same seed lot in the same or different laboratories (P=0.05) (appropriate for comparing pure seed determined by the fluorescence test for ryegrass, the copper sulfate-ammonia test for sweet clover, or tests for separation of species based on morphological characteristics).

No. of seeds in the second test to which tolerance is to be applied	400	400	800	800	400	800	1000	1000
No. of seeds in the first test (or the test used to generate the label)	400	800	400	800	1000	1000	1000	800

No change in the tolerance values of Table 13H, only the title and the left column headings will be changed.

#

Harmonization and impact statement: The Federal Seed Act follows the current AOSA Rule as stated above. The International Seed Testing Association does not have this table. The Canadian Food Inspection Agency Methods, Procedures for Testing Seeds follows the current AOSA Rule as stated above.

Supporting evidence: The proposed change will make it easier to understand and apply Table 13H not only on how to determine the percentage of pure seed based on the fluorescence test for ryegrass, but also for the chemical test of sweet clover and the separation of species based on morphological characteristics. The proposed change will also provide examples on how to calculate the interpolation.

Submitted by: Sabry G. Elias, Oregon State University Seed Laboratory, (541) 737 4464; and Deborah Meyer, California Department of Food and Agriculture, (916) 262-1137

Date Submitted: October 15, 2010; Revised January 10, 2011

2011 Rules Change Proposal 26

Purpose of Proposal: To add *Pittosporum undulatum* to the Uniform Classification Table of the AOSA Rules.

Present Rule: New rule

Proposed Rule:
AOSA Rules for Testing Seeds Volume 3. Uniform Classification of Weed and Crop Seeds -- Uniform Classification Sorted by Scientific Name:

NOMEN #	SCIENTIFIC NAME	COMMON NAME	FAMILY	SPP. CLASS	CONTAMINATING CLASSIFICATION						
					A	F	H	R	S	T	V
28757	*Pittosporum undulatum* Vent.	Box, Victorian	Pittosporaceae	S	W	W	W	W	W	W	W

Harmonization and Impact Statement: This proposal does not harmonize with ISTA, Canadian methods, or the Federal Seed Act. ISTA classifies all contaminating species as other seed; the Federal Seed Act leaves the classification of contaminants up to the individual states; and *Pittosporum undulatum* is not considered a noxious weed in Canada.

Supporting Evidence: *Pittosporum undulatum* (Victorian box) is listed on the 2010 State Noxious-Weed Seed Requirements Recognized in the Administration of the Federal Seed Act as a noxious weed seed in Hawaii. Because it is noxious, this species should be included on the Uniform Classification Table of the AOSA Rules. Victorian box is a tree native to Australia and sometimes used as a landscape plant; it is considered an invasive species in some areas.

References:

Bailey, LH (ed.) 1976. Hortus Third – A Concise Dictionary of Plants Cultivated in the United States and Canada. Macmillan Publishing Company.

Germplasm Resources Information Network (GRIN):
http://www.ars-grin.gov/cgi-bin/npgs/html/tax_search.pl

Submitted By:

Michael Aberle, R.S.T
Sue Alvarez, R.S.T.
Ransom Seed Laboratory
P.O. Box 300
Carpinteria, California 93014-0300
Phone: 805-684-3427
Fax: 805-684-4157
Email: RansomSL@silcom.com

Date Submitted: October 15, 2010