

## Rule Change Proposal No. 10

**PURPOSE OF PROPOSAL:** To add purity and germination test procedures for *Puccinella distans*, weeping alkaligrass, to the Rules.

### PRESENT RULE

Handbook 25

SCIENTIFIC / COMMON NAME	FAMILY	SPP. CLASS	CONTAMINATING CLASSIFICATION						
			A	F	H	R	S	T	V
<i>Puccinella distans</i> ---alkaligrass, weeping	Poaceae	R	W	W	W	C	W	C	W

### PROPOSED RULE

The Handbook 25 classification scheme will remain unchanged.  
Seedling evaluation will follow the guidelines for Poaceae, Grass Family V – Other Kinds.

#### Section 2.4, Table 1. 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
23	<i>Puccinella distans</i> (Jacq.) Parl. weeping alkaligrass	0.6	6	4,107	116,424

#### 2.7 e. Pure seed unit (PSU) definitions

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

## 2.11 Uniform blowing procedure

- b. **Purpose:** The uniform blowing procedure shall be used for separation of pure seed and inert matter in the following: Kentucky bluegrass (*Poa pratensis*), Canada bluegrass (*P. compressa*), rough bluegrass (*P. trivialis*), alkaligrass (*Puccinella distans*), Pensacola variety of bahiagrass (*Paspalum notatum*), orchardgrass (*Dactylis glomerata*), blue grama (*Bouteloua gracilis*), and side-oats grama (*B. curtipendula*).
- d. **Procedures:** The size of the samples to be blown shall be the same as that for a purity test except for blue grama and side-oats grama, which shall be divided into four approximately equal parts prior to blowing. All seed kinds are to be blown for 3 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 will be considered inert matter. Remove all weed and crop seeds and other inert matter (stems, leaves, dirt) from the heavy portion and add these to the weed, crop or inert matter separations, as appropriate. The remainder of the heavy portion will be considered pure seed.

When kinds listed in this section appear in mixtures, they shall be separated from other kinds before using the uniform blowing procedure.

- (8) **Weeping alkaligrass:** The blower setting obtained for the Kentucky bluegrass calibration sample multiplied by a factor of 0.76 shall be used. (The 0.76 factor is restricted to the General-type Seed Blower, see section 3.3(a) in AOSA Handbook No. 24.)

### Section 4.10, Table 3. Methods of testing for laboratory germination.

Kind of Seed	Substrata	Temperature °C	First count days	Final count days	<u>Additional Directions</u> See Sec. 4.2 and 4.9
<i>Puccinella distans</i> weeping alkaligrass	P	15-25	14	21	Light, KNO <sub>3</sub>

## HARMONIZATION

Weeping alkaligrass is not in the Federal Seed Act or the ISTA Rules. This species is listed in the Canadian Methods and Procedures. Under Table 1 of the Canadian Methods and Procedures the minimum working weight for a purity test, if required, is 1 gram. The purity testing method requires hand separation and checking for the presence of a caryopsis. Germination requirements are similar to those proposed, except the final count is at 28 days and prechill is recommended for fresh and dormant seeds.

## SUPPORTING EVIDENCE

Research to determine the appropriate working weight and to compare the hand separation method for purity analysis to several uniform blowing point factors was conducted at the California State Seed Laboratory. Germination procedures used were similar to those described by Chirco and Turner (1986). A second study was conducted among nine laboratories to compare the hand separation method to the blowing point method determined to compare best to the hand separation method in the initial study. The two methods produced nearly identical results and the uniform blowing point method required approximately 42% less time than the hand separation method. This work is detailed in Meyer and Effenberger (2002).

Examples of seed units of weeping alkaligrass are shown in Figure 1. Data in tables 1 – 3 were generated in a study comparing different uniform blowing methods with the hand separation method for purity testing. The General Blower settings were based on factors of the Kentucky bluegrass blowing point. Seed from the heavy fraction was subsequently germinated and pure live seed percentages were calculated. This work was conducted at the California State Seed Laboratory.

Table 1. Seed counts of 22 lots of weeping alkaligrass following the procedure in Appendix 4 of the AOSA Rules.

Lot #	Mean Wt. (gm) per 100 seed	Mean # seeds/gm	Mean # Seeds/oz	Minimum Purity Working Wt. (gm)	Minimum Noxious Working Wt. (gm)
A	0.0235	4255	120,629	0.6	6
B	0.0235	4255	120,629	0.6	6
C	0.0249	4016	113,854	0.6	6
D	0.0242	4132	117,142	0.6	6
E	0.0225	4444	125,987	0.6	6
F	0.0233	4292	121,678	0.6	6
G	0.0241	4149	117,624	0.6	6
H	0.0279	3584	101,606	0.7	7
I	0.0304	3289	93,243	0.8	8
J	0.0225	4444	125,987	0.6	6
K	0.0231	4329	122,727	0.6	6
L	0.0219	4566	129,446	0.5	5
M	0.0224	4464	126,554	0.6	6
N	0.0211	4739	134,351	0.5	5
O	0.0249	4016	113,854	0.6	6
P	0.024	4167	118,134	0.6	6
Q	0.0287	3484	98,771	0.7	7
R	0.0227	4405	124,882	0.6	6
S	0.0253	3953	112,068	0.6	6
T	0.0277	3610	102,344	0.7	7
U	0.0262	3817	108,212	0.7	7
V	0.0254	3937	111,614	0.6	6
<b>Mean</b>	<b>0.0246</b>	<b>4107</b>	<b>116,424</b>	<b>0.6</b>	<b>6</b>

**Figure 1.** Examples of pure seed units of alkaligrass. A and C – fertile floret with glumes and pedicel; B – fertile floret; D – three attached florets (two fertile, one sterile).



**Table 2.** Mean percentages of pure seed and pure live seed (PLS), and variances (V) across 12 lots of weeping alkaligrass for six purity methods.

Method	% Pure Seed	V	% PLS	V
HSM	98.81	1.39	88.86	37.25
UBP 7.6	98.95	1.53	90.55	32.64
UBP 8.2	98.74	1.75	91.30	24.16
UBP 8.8	98.53	2.24	90.75	52.44
UBP 9.4	98.39	4	91.09	40.29
UBP 10.0	98.15	4.43	90.94	35.21

**Table 3.** Comparisons of mean differences of percent pure seed and percent pure live seed (PLS) between hand separation method (HSM) and five uniform blowing methods (UBP).

Methods	Mean diff. between percent pure seed	Mean diff. between percent pure live seed
HSM – UBP 7.6	-0.14	-1.69
HSM – UBP 8.2	0.07	-2.47
HSM – UBP 8.8	0.28	-1.89
HSM – UBP 9.4	0.42	-2.23
HSM – UBP 10.0	0.66	-2.08

Based on the results of this study, a blowing point factor of 0.76 was selected for further referee testing among laboratories. Nine laboratories participated in the collaborative study to compare purity testing methods of hand separation and uniform blowing for five lots of weeping alkaligrass. Data enumerated in tables 4 – 6 summarize this study.

**Table 4.** Percentage of pure seed and pure live seed from the Hand Separation Method (HSM) and the Uniform Blowing Procedure (UBP) within and across lots, least squares means, standard error and F-value for comparison between test methods.

Lot	Mean % Pure Seed		Mean % Pure Live Seed	
	HSM	UBP	HSM	UBP
1	99.49	99.56	94.18	94.80
2	98.95	99.33	93.56	93.81
3	97.76	97.61	90.27	91.21
4	99.28	99.03	93.65	94.85
5	99.55	99.51	94.79	95.53
LS Means	99.0047	99.0062	93.2916	94.0420
Standard Error	0.3504	0.3521	1.0865	1.0693
F-value	0		5.36†	

† significant at 0.05 level.

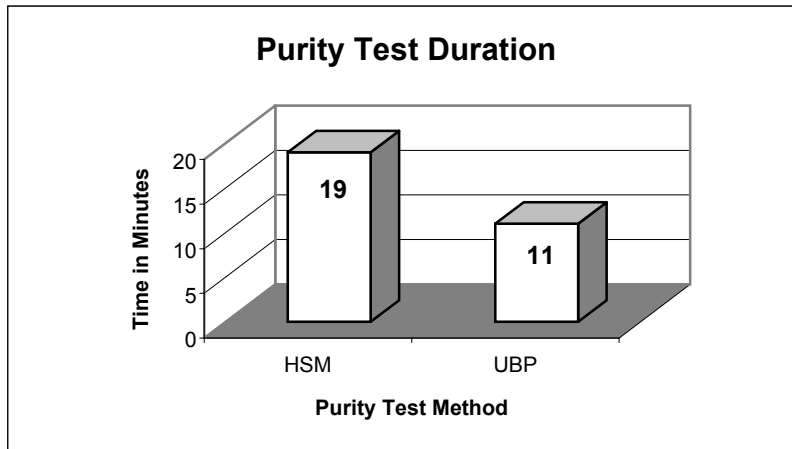
**Table 5.** Covariance Parameter Estimates of pure seed percentages and PLS percentages ( $p = 0.05$ ).

Covariance Parameter	Group	Pure Seed				PLS			
		Estimate of Covariance	SE	Lower limit	Upper limit	Estimate of Covariance	SE	Lower limit	Upper limit
UN (1,1)	UBP	0.127	0.039	0.075	0.260	1.529	0.570	0.826	3.744
UN (1,1)	HSM	0.072	0.033	0.035	0.227	3.195	0.826	2.039	5.713

**Table 6.** Comparison of mean laboratory results for percentages of pure seed (PS), germination (G) and pure live seed (PLS) across all lots and methods. Means within test type with the same letter are not significantly different ( $p = 0.05$ ). High and low means within each test type are underlined.

Lab	Mean % PS	Mean % G	Mean % PLS
1	99.17 b	92.40 e	91.65 e
2	98.89 bc	96.50 ab	95.43 ab
3	98.90 bc	95.20 bcd	94.16 bcd
4	98.93 bc	<u>89.70</u> f	<u>88.75</u> f
5	98.97 bc	94.10 d	93.14 ed
6	<u>99.54</u> a	<u>97.10</u> a	<u>96.65</u> a
7	98.93 bc	94.70 dc	93.70 cd
8	<u>98.74</u> c	95.90 abc	94.70 bc
9	98.98 bc	95.80 abc	94.83 bc

**Figure 2.** Comparison of mean times among laboratories to complete purity tests of alkaligrass using the hand separation method (HSM) and the Uniform Blowing Procedure (UBP).



Among the nine participating laboratories, results for the two purity methods across five seed lots were essentially the same and the pure live seed results were very close. There was a significant decrease in mean purity test time with the uniform blowing point method (Figure 2).

References:

Chirco, E.M. and T.L. Turner. 1986. Species without AOSA testing procedures. Association of Official Seed Analysts News Letter 60:2-70.

Meyer, D.J.L. and J. Effenberger. (2002). Comparison of Purity Testing methods of Weeping Alkaligrass (*Puccinella distans* (Jacq.) Parl.). Submitted to Seed Technology.

**SUBMITTED BY**

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