

## Rules Change Proposal – 14 - Amended

**Purpose:** To modify the current purity analysis and germination testing methods for samples of coated or encrusted kinds from Poaceae.

### Present and proposed rule:

#### 3.8 Pelleted, coated or encrusted seed purity procedures

e. **The purity analysis of coated seed** (this section does not apply to seed samples that are single component seed samples of Poaceae, or any mixtures of kinds):

(1) Separation of component parts: The working sample shall be weighed in grams to the appropriate number of decimal places (refer to section 2.3) and shall be separated into four parts:

- (a) Pure coated units as defined in 3.8(2).
- (b) Uncoated crop seed as defined in 3.8(3) (including the kind under consideration).
- (c) Inert matter as defined in 3.8(4).
- (d) Uncoated weed seed as defined in 3.8(5).

(2) Pure coated units shall include:

- (a) Entire coated units regardless of whether or not they contain a seed.
- (b) Broken and damaged coated units in which more than half the surface of the seed is covered by coating material, except when it can be seen that, either the seed is not of the species stated by the sender, or there is no seed present.

(3) Uncoated crop seed shall include:

- (a) Free seeds of any crop species; refer to sections 3.2 and 3.3.
- (b) Broken coated units containing a crop seed that is recognizably not of the species stated by the sender.
- (c) Broken coated units of the species stated when the coating material covers half or less of the surface of the seed.

(4) Inert matter shall include:

- (a) Loose coating material.
- (b) Broken coated units in which it is obvious there is no seed.
- (c) Any other material defined as inert matter in section 3.5.

(5) Uncoated weed seed shall include:

- (a) Free seeds of any weed species; refer to section 3.4.
- (b) Broken coated units containing a weed seed.

f. **Purity analysis of de-coated seed**, ~~to be performed upon request or if necessary because the sample is a mixture~~ (This section shall apply to all mixtures of kinds, single component seed samples of Poaceae, or upon request for other kinds):

(1) Determine the working sample size as in section 2.3 b (5), and weigh the working sample in grams to the appropriate number of decimal places (refer to section 2.3 a).

- (2) Any loose coating material shall be sieved, weighed, and included with the inert matter component.
- (3) Remove the coating material from the seed by washing with water or other solvents such as, but not limited to, dilute sodium hydroxide. Use of fine mesh sieves is recommended for this procedure, and stirring or shaking the coated units may be necessary to obtain de-coated seed.
- (4) Spread on blotters or filter paper in a shallow container. Air dry overnight at room temperature.
- (5) Separation of component parts:
  - (a) Kind or cultivar considered pure seed [as defined in sec. 3.2 and Table 3A](#).
  - (b) Other crop seed.
  - (c) Inert matter.
  - (d) Weed seed.
  - (e) Coating material.

The de-coated seed shall be separated into the first four components in accordance with sections 3.2 through 3.5. Sections 3.6 and 3.7 shall not be followed. The weight of the coating material component is determined by subtracting the sum of the weights of the other four components from the original weight of the working sample. Calculate percentages of all five components based on the original weight of the working sample.

Table 3A Pure seed unit definitions

PSU Number	Description of Pure Seed Unit
22	<p>Multiple floret spikelet, multiple floret, or floret, with or without pedicel, with or without awn(s), provided there is a caryopsis at least one-third the length of the palea measured from the base of the rachilla.</p> <p>Caryopsis or piece of broken caryopsis larger than one-half of the original size.</p> <p>The amount of inert matter attached to the multiple units shall be determined by the method described in section 3.7.</p> <p><u>Special consideration:</u></p> <ul style="list-style-type: none"> <li>• <a href="#">When coated seed units are de-coated for purity analysis the method in sec. 3.7 shall not be used. Separation of multiple units shall be as follows:</a></li> <li>• <a href="#">A fertile floret attached to another fertile floret shall be separated.</a></li> <li>• <a href="#">Attached glumes and empty florets extending to or beyond the tip of the fertile floret shall be removed and classified as inert matter.</a></li> </ul>

23	<p>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.</p> <p>Special consideration:</p> <ul style="list-style-type: none"> <li>• For <i>Bouteloua curtipendula</i>, in addition to the units described above, spikelet group that disarticulates as a unit with attached rachis and internode.</li> <li>• <a href="#">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.</a></li> </ul>
24	<p>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. After the Uniform Blowing Point Procedure is completed, the amount of inert matter attached to the multiple units shall be determined by the method described in section 3.7.</p> <p><a href="#">Special consideration:</a></p> <ul style="list-style-type: none"> <li>• <a href="#">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.</a></li> <li>• <a href="#">When coated seed units are de-coated for purity analysis the method in sec. 3.7 shall not be used. Separation of multiple units shall be as follows:</a> <ul style="list-style-type: none"> <li>• <a href="#">A fertile floret attached to another fertile floret shall be separated.</a></li> <li>• <a href="#">Attached glumes and empty florets extending to or beyond the tip of the fertile floret shall be removed and classified as inert matter.</a></li> </ul> </li> </ul>

## 6.8 Special procedures and alternate methods for germination

**l. Coated seed.** — Where reference is made to coated seed the rules also apply to pelleted and encrusted seed. Refer to section 2.1 b.

(1) Germination tests on coated seed units and on de-coated seed shall be conducted in accordance with methods in Table 6A. Kinds for which soaking or washing is specified in section 6.8 shall not be soaked or washed in the case of coated seed. For coated seed pleated paper may be used.

(a) Coated seed units shall be placed on the substratum in the condition in which they are received without rinsing, soaking, or any other pretreatment, [unless the sample was decoated for purity analysis in accordance with 3.8.f.](#)

(b) ~~Coated seed units in mixtures that are color coded or can otherwise be separated by kinds shall be germinated as separate kinds without removing the coating material.~~ [When a purity analysis is not conducted on kinds from Poaceae, the coating must be removed before planting to identify the pure seed units. Remove the coating material in a manner that will not affect the germination of the seeds and plant after air drying at room temperature for not more than 72 hours. Refer to sec. 3.8.f\(3\) for coating removal method.](#)

- (c) Coated seed units in mixtures that cannot be separated by kinds without removing the coating material shall have the coating removed in a manner that will not affect the germination capacity of the seeds. The de-coated seeds shall be planted as separate kinds ~~on the same day the coating material is removed~~ after air drying at room temperature for not more than 72 hours.
- (d) On request or as a comparison, germination may be made on de-coated seed of any kind. Remove the coating material in a manner that will not affect the germination of the seeds and plant ~~the same day~~ after air drying at room temperature for not more than 72 hours. Refer to sec. 3.8.f (3) for coating removal method.
- (2) The moisture level of the substratum is important. It may depend on the water-absorbing capacity of the coating material. A retest may be necessary before satisfactory germination of the sample is achieved.
- (3) Phytotoxic symptoms may be evident when germinating coated seeds in paper substrata. In such cases a comparative test or retest in sand or soil may be necessary. For pelleted or film-coated onion, see section 6.8 r.

**Harmonization:** This change will bring the testing of the coated and encrusted grass species stated above in compliance with the Federal Seed Act Regulations sec. 201.51b. This section requires the removal of coating material for purity analysis for all kinds of seed marketed as coated products. This proposal will also harmonize with germination test requirements under Federal Seed Act Regulations sec. 201.58(c)(1)(iii) for seed mixtures that cannot be separated by kinds without removing the coating material. According to the Federal Seed Act Regulations sec. 201.58(c)(1)(i), single component kinds, including members of Poaceae, require coated seed units to be tested in the condition in which they are received without rinsing, soaking or any other pretreatment.

**Supporting Evidence:** The rule will help correct labeling issues related to the labeling of coated (including encrusted) grass seed products that are being offered in the marketplace. If seed lots of these products are tested and labeled using the method under 3.8.e they will be in violation of the Federal Seed Act. Note: further supporting evidence ~~will be submitted when is included in~~ the referee on coated grass seed is completed in the following appendix.

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## **Referee Study: Differences in Purity and Germination Test Results Among Coated and De-Coated Grass Samples**

This study was a follow up to the tentative rule proposal, recently approved, to modify the current purity analysis and germination testing methods for samples of coated or encrusted kinds from Poaceae. Laboratories were asked to test different coated grass mixtures and compare purity and germination results with and without a de-coating step. Germination of coated seeds from single component samples was compared to germination of the same samples after de-coating and drying for 1, 2 or 3 days. Eight seed testing labs participated in this referee study. The specific objectives were to:

- **Objective 1.** Determine if de-coating of mixtures is a necessary step in purity analysis, and the extent of differences in results between coated and de-coated samples.
- **Objective 2.** Determine if germination results significantly differ when seeds from the same lot are tested as coated or de-coated.
- **Objective 3.** Determine the effect of different drying periods after de-coating, up to 3 days.

### **Procedures**

#### **I. Purity and germination testing of coated versus de-coated grass seed mixtures**

- For this test, three mixtures were used. Two mixtures had ryegrass and Kentucky bluegrass as the main components, and one mixture had tall fescue and Kentucky bluegrass as the main components. From each sample two identical working samples were derived, for a total of six working samples. Each working sample was labeled for analysis as either coated or de-coated, but no other information as to origin or composition was provided to analysts. The working sample weights for each mixture were based on multiple 100-seed counts, and contained at least 2500 seeds. For purity analysis, the whole working sample was used. The procedure was as follows:
  1. For three of the above samples, labeled Coated Analysis, purity was tested without de-coating. The working samples were weighed and the component parts separated into pure coated units, uncoated crop seed, inert matter and uncoated weed seed according to AOSA rules (3.8 e). The different components were weighed to the nearest 0.001 gram. No weed seed identification was required.
  2. For the other three working samples, representing duplicates of the first three samples but labeled De-coated Analysis, seeds were de-coated and then tested for purity according to AOSA rules. The procedure was as follows:
    - a. Working samples were weighed then the coating material removed as described in AOSA rules (3.8 f 3). This was accomplished by washing with water for a period not to exceed 30 minutes. Use of fine mesh sieves was recommended for this procedure along with gentle stirring or shaking to obtain almost completely de-coated seed, a necessary condition for this study.
    - b. De-coated seeds were spread on blotter or filter paper at room temperature to dry for 30-60 minutes. Wet blotter/filter papers were then replaced with dry ones, and

the drying process continued overnight. The elapsed time from the start of the drying period, followed by purity analysis, to the initiation of the germination test did not exceed 24 hours.

- c. The component parts were separated and weighed into pure seed, other crop seed, inert matter and weed seed as described in AOSA rules (3.8 f 5). The weight of the coating material component was determined by subtracting the weights of the above components from the total initial weight of the sample. The different components were weighed to the nearest 0.001 gram. Weed seed identification was not required.
3. Following the purity test, germination tests were performed on the resultant pure seed components from all samples, coated and de-coated. As mentioned above for de-coated seed, the elapsed time from the start of the drying period to the initiation of the germination test did not exceed 24 hours. All seeds were germinated on top of a triple layer of white filter paper in a covered box. For each germination test, 400 seeds in 4 replicates were used. Appropriate temperature, test duration, count days and other conditions were based on AOSA rules for each kind tested.

## **II. Germination of de-coated pure seeds following different drying periods**

Six single component samples of coated Bermudagrass (2 samples), Kentucky bluegrass (two samples), tall fescue and fine fescue were tested. The procedure was as follows:

1. Germination of coated seeds: Four replicates of 100 seeds each were planted as is (*coated*) without any further treatments. Seeds were germinated on top of a triple layer of white filter paper in a covered box. AOSA rules were followed in determining appropriate temperature, test duration, count days and other conditions for each kind tested.
2. De-coating: all the remaining seeds of the working sample were then de-coated as follows:
  - a. The coating material was removed as described in AOSA rules (3.8 f 3). This was accomplished by washing with water for a period not exceeding 30 minutes. Use of fine mesh sieves was recommended for this procedure along with gentle stirring or shaking to obtain almost completely de-coated seed, a necessary condition for this study.
  - b. De-coated seeds were then spread on blotter or filter paper at room temperature to dry for 30-60 minutes. Wet blotter/filter papers were then replaced with dry ones, and the drying process continued.
3. De-coated/1 day drying: After a drying period of 18-24 hours, 400 seeds were randomly selected and germinated following AOSA rules as described above. For this sub-sample, the drying period did not exceed 24 hours.
4. De-coated/2 days drying: The drying process was continued for the remaining seeds. After a drying period of 42-48 hours, another sample of 400 seeds was randomly selected and germinated following AOSA rules as described above. For this sub-sample, the drying period did not exceed 48 hours.
5. De-coated/3 days drying: The drying process was continued for the remaining seeds. After a drying period of 66-72 hours, another sample of 400 seeds was randomly selected and germinated following AOSA rules as described above. For this sub-sample, the drying period did not exceed 72 hours.

## Results and Data Analysis

Data from participating laboratories was collected and analyzed. Germination data was transformed to arc-sine values before statistical analysis. Variation was estimated based on several parameters and appropriate tolerance tables were used to evaluate differences among treatments, both for purity and germination tests. Analysis of variance was used to evaluate the extent of overall variation as well as the observed variation for each type of treatment. The test results are summarized in tables 1-5.

**Table 1. Coated/Decoated analysis: Percent composition; Ryegrass-Kentucky bluegrass mixture 1.**

	Mean	Minimum	Maximum	Std. Deviation	CV (%)
<b>Coated analysis</b>					
Ryegrass	78.478	76.992	80.787	1.32	1.7
Kentucky bluegrass	20.126	17.753	21.702	1.27	6.3
Uncoated Crop Seed	0.298	0.000	0.647	0.190	63.7
Inert matter	1.093	0.594	2.095	0.518	47.4
Weeds	0.005	0.000	0.040	0.0150	>100
<b>Decoated analysis</b>					
Ryegrass	42.258	40.508	46.655	2.148	5.0
Kentucky bluegrass	8.412	7.622	8.851	0.438	5.2
Other crop seed	0.139	0.000	0.298	0.104	74.0
Inert matter	0.698	0.206	1.023	0.295	42.0
Weeds	0.009	0.000	0.040	0.017	>100
Coating materials	48.484	44.279	54.144	2.990	6.1

Ratio Ryegrass/Kentucky bluegrass; coated sample: 3.90

Ratio Ryegrass/Kentucky bluegrass; decoated sample: 5.02

**Table 2. Coated/Decoated analysis: Percent composition; Ryegrass-Kentucky bluegrass mixture 2.**

	Mean	Minimum	Maximum	Std. Deviation	CV (%)
<b>Coated analysis</b>					
Ryegrass	74.789	71.804	77.688	1.976	2.6
Kentucky bluegrass	23.377	21.126	24.860	1.292	5.5
Uncoated Crop Seed	0.197	0.000	0.451	0.156	79.4
Inert matter	1.637449	.7042	3.1463	1.0174507	62.1
Weeds	0.000	0.000	0.000	0.000	-
<b>Decoated analysis</b>					
Ryegrass	40.119	37.634	44.794	2.407	6.0
Kentucky bluegrass	10.839	10.415	11.689	0.482	4.4
Other crop seed	0.100	0.000	0.221	0.072	71.9
Inert matter	1.266	0.433	4.875	1.602	>100
Weeds	0.000	0.000	0.000	0.000	-
Coating materials	47.677	42.662	51.189	3.503	7.3

Ratio Ryegrass/Kentucky bluegrass; coated sample: 3.20

Ratio Ryegrass/Kentucky bluegrass; decoated sample: 3.70

**Table 3. Coated/Decoated analysis: Percent composition; Tall fescue-Kentucky bluegrass mixture.**

	Mean	Minimum	Maximum	Std. Deviation	CV (%)
<b>Coated analysis</b>					
Tall fescue	52.683	49.940	54.008	1.337	2.5
Kentucky bluegrass	45.952	44.983	47.091	0.808	1.8
Uncoated Crop Seed	0.0758	0.000	0.177	0.0714	94.2
Inert matter	1.289	0.745	2.969	0.798	61.9
Weeds	0.000	0.000	0.000	0.000	-
<b>Decoated analysis</b>					
Ryegrass	28.132	26.305	29.261	1.130	4.0
Kentucky bluegrass	26.027	24.452	27.447	1.112	4.3
Other crop seed	0.047	0.000	0.174	0.063	>100
Inert matter	0.631	0.394	0.925	0.206	32.7
Weeds	0.003	0.000	0.017	0.006	>100
Coating materials	45.161	42.906	46.646	1.294	2.9
Ratio Tall fescue/Kentucky bluegrass; coated sample: 1.15					
Ratio Tall fescue/Kentucky bluegrass; decoated sample: 1.08					

**Table 4. Coated/Decoated analysis: Variation among lab results for each pure seed component (% composition of total sample) of various grass mixtures.**

Component	Sample					
	Ryegrass-Kentucky bluegrass mixture 1		Ryegrass-Kentucky bluegrass mixture 2		Tall fescue-Kentucky bluegrass mixture	
	Ryegrass	Kentucky bluegrass	Ryegrass	Kentucky bluegrass	Tall fescue	Kentucky bluegrass
<b>Coated seeds</b>						
<b>Laboratory</b>						
<b>1</b>	79.725ab	19.350ab	75.169bcd	23.578ab	54.008b	44.983a
<b>2</b>	77.869a	20.850b	73.548abc	24.859b	52.783ab	46.108a
<b>3</b>	80.787b	17.753a	76.088bcd	23.177ab	53.872b	45.010a
<b>4</b>	78.248ab	20.422b	73.455ab	23.398ab	49.940a	47.091a
<b>5</b>	76.992a	21.702b	77.688d	21.126a	52.700ab	46.555a
<b>6</b>	77.548a	20.097ab	71.804a	24.844ab	52.834ab	45.508a
<b>7</b>	78.177ab	20.705b	75.772bcd	22.654ab	52.643ab	46.413a
<b>De-coated seeds</b>						
<b>Laboratory</b>						
<b>1</b>	42.494a	8.739a	39.427ab	11.269a	27.506a	26.396a
<b>2</b>	40.508a	8.355a	39.277ab	10.467a	26.305a	27.087a
<b>3</b>	46.655b	8.771a	44.794c	11.689a	29.182a	27.447a
<b>4</b>	42.407a	8.851a	41.372bc	10.907a	29.261a	26.171a
<b>5</b>	41.300a	8.818a	37.634a	10.568a	27.991a	24.452a
<b>6</b>	41.333a	8.624a	38.125ab	10.557a	27.490a	25.885a
<b>7</b>	41.107a	7.922a	40.200ab	10.415a	29.189a	24.752a

Means in each column, for each component, with the same letter, are within tolerance of each other (Table 13A, AOSA Rules).

**Table 5. Germination results; grass mixtures.**

<b>Component</b>	<b>% germination</b>	<b>Significance</b>
<b>Ryegrass-Kentucky bluegrass mixture 1</b>		
Kentucky bluegrass coated	83	NS*
Kentucky bluegrass decoated	83	
Ryegrass coated	91	NS
Ryegrass decoated	91	
<b>Ryegrass-Kentucky bluegrass mixture 2</b>		
Kentucky bluegrass coated	62	NS
Kentucky bluegrass decoated	56	
Ryegrass coated	67	NS
Ryegrass decoated	67	
<b>Tall fescue-Kentucky bluegrass mixture</b>		
Kentucky bluegrass coated	86	NS
Kentucky bluegrass decoated	84	
Tall fescue coated	89	NS
Tall fescue decoated	91	

\*NS: not significant

**Table 6. Germination results of single component seed samples.**

Lab #	TRT	SAMPLE					
		Kentucky bluegrass 1	Kentucky bluegrass 2	Bermudagrass 1	Bermudagrass 2	Fine fescue	Tall fescue
1	Coated	87	86	91	87	84	86
	Decoated-1 day	87	86	87	92	85	89
	Decoated-2 days	88	88	89	90	86	86
	Decoated-3 days	86	85	87	88	85	87
2	Coated	85	81	93	88	90	89
	Decoated-1 day	87	83	92	90	87	92
	Decoated-2 days	84	86	90	89	89	93
	Decoated-3 days	89	81	90	90	91	93
3	Coated	89	77	91	90	83	86
	Decoated-1 day	90	68	94	91	89	84
	Decoated-2 days	88	73	93	94	93	88
	Decoated-3 days	89	65	95	89	84	91
4	Coated	85	81	89	85	88	86
	Decoated-1 day	88	82	90	95	90	92
	Decoated-2 days	86	84	93	95	88	91
	Decoated-3 days	88	82	92	92	90	88
5	Coated	86	75	88	90	87	89
	Decoated-1 day	82	74	79	82	88	87
	Decoated-2 days	82	71	92	87	81	89
	Decoated-3 days	87	72	88	90	86	86
6	Coated	85	80	92	94	88	88
	Decoated-1 day	81	78	90	90	89	89
	Decoated-2 days	84	81	89	90	87	89
	Decoated-3 days	83	82	89	90	86	-
7	Coated	86	85	88	92	88	86
	Decoated-1 day	84	79	92	90	84	86
	Decoated-2 days	84	84	89	90	84	85
	Decoated-3 days	82	81	93	94	84	92
Mean	Coated	86	81	90	89	87	87
	Decoated-1 day	86	79	89	90	87	88
	Decoated-2 days	85	81	91	91	87	89
	Decoated-3 days	86	78	91	90	87	90

\*None of the differences, within each laboratory, were significant among the different treatments (coated, decoated 1-day, de-coated 2 days, and de-coated 3 days)

Based on the above results, the study's objectives were met as follows:

**Objective 1.** Determine if de-coating of mixtures is a necessary step in purity analysis, and the extent of differences in results between coated and de-coated samples.

As stated in the proposal, and supported by results from the referee test, de-coating is a necessary step in purity analysis. The extent of differences in purity analysis results (Tables 1-3), especially the differences in the ratio of pure seed components of coated versus de-coated samples, indicates the necessity of de-coating grass seed samples for purity analysis. Variation among laboratories (Table 4) was also significant, with many significant differences among labs for single component results.

**Objective 2.** Determine if germination results significantly differ when seeds from the same lot are tested as coated or de-coated.

Results from mixtures (Table 5) indicated no differences in germination between coated and un-coated seeds for any of the components tested. Additionally, none of the differences among laboratories, for each component, were significant (data not shown).

**Objective 3.** Determine the effect of different drying periods after de-coating, up to 3 days.

Drying period ranging from 1 to 3 days had no effect on germination results, and none of the differences were found to be significant (Table 6). Additionally, none of the differences among laboratories, for each treatment, were significant (data not shown).