RULE PROPOSALS - 1989

AOSA Rules Committee
Stephen J. Hurst, Chairman
Federal Seed Laboratory
Bldg. 306, Rm. 213, BARC-East
Beltsville, Maryland 20705-2325

The following proposals for changes in or additions to the AOSA Rules For Testing Seeds have been evaluated and approved by the Rules Committee. These proposals will be voted on by the AOSA membership at their 1989 business meeting in Illinois.

Please read and review all proposals along with reasons and supporting evidence. Comments concerning content and/or wording of these proposals are welcome and can be forwarded to either the Rules Committee Chairman or Mark Johnson, (5550 NW 55th Avenue, Johnston, Iowa 50131), the SCST liaison representative on the Rules Committee. Your comments will be presented and discussed at the Open Rules Committee meeting in June.

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1. PROPOSAL

Addition of peroxidase test for soybean (Glycine max) to section 3.2 Identification and Cultivar Determination.

PROPOSED RULE

3.2.b
(2) Peroxidase Test for Soybean (Glycine max): Remove and place the dry seed coat from at least 400 soybean seeds into individual test tubes or suitable containers. Add 10 drops (0.5-1.0 ml) of 0.5 percent guaiacol to each test tube. After waiting 10 minutes add one drop (about 0.1 ml) of 0.1 percent hydrogen peroxide. One minute after adding the hydrogen peroxide, record the seed coat as peroxidase positive (high peroxidase activity) indicated by a reddish-brown solution or peroxidase negative (low peroxidase activity) indicted by a colorless solution in the test tube.

BACKGROUND INFORMATION AND SUPPORTING EVIDENCE

Background information: Buttery and Buzzell (1968) were able to separate soybean cultivars into two groups based on the presence of either high or low seed coat peroxidase activity. A genetic analysis, by Buzzell and Buttery (1969) revealed that seed coat peroxidase activity is controlled by a major gene. They found that high activity results from the presence of the dominant allele while low activity results from the presence of homozygous recessive alleles.

Supporting evidence: Experiments by Payne (1976) indicate that the outcome of the seed coat peroxidase test is unaffected by seed quality or seed storage conditions.

Referee tests conducted by the Federal Seed Laboratory and the 1981-82 AOSA/SCST Referee Project for the Southern Region confirmed that the results of the soybean seed coat peroxidase test are consistently reproducible by different laboratories.

The procedure listed in the proposed rule is the same procedure that was used successfully in the referee tests. This procedure is also included on pages 23 and 24 of the Progress Report on the AOSA Cultivar Purity Testing Handbook published as Volume 62, No. 3 of the AOSA Newsletter in May 1988.

The soybean seed coat peroxidase test is currently being used by a number of seed testing laboratories to help confirm that seed lots are correctly labeled as to cultivar and to determine if certain seeds are contaminates. Therefore, this testing procedure should be incorporated into the AOSA Rules.

LITERATURE CITED

Buttery, B.R., and R.I. Buzzell. 1968. Peroxidase activity in seeds of soybean varieties. Crop Sci. 8:723-725.

Buzzell, R.I., and B.R. Buttery. 1969. Inheritance of peroxidase activity in soybean seed coats. Crop Sci. 9:387-388.

Payne, R.C. 1976. Seed coat peroxidase activity as a aid in differentiating soybean cultivars. AOSA Newsletter 50(1):43-45.

Payne, R.C. 1979. Some new tests and procedures for determining variety (soybeans). J. Seed Technol. 3:61-77.

Staff (Louisiana State Seed Testing Laboratory). 1978. Phenotypic characteristics of thirty-two soybean cultivars grown in Louisiana and their peroxidase activity. AOSA Newsletter 52(1):41-43.

SUBMITTED BY

Richard C. Payne, Federal Seed Laboratory, USDA, AMS, Beltsville, MD. 20705

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2. PROPOSAL

Addition of fluorescence test for oat (Avena sativa) to section 3.2 Identification and Cultivar Determination.

PROPOSED RULE

3.2.c
Fluorescence Test for Oat (Avena sativa): Place at least 400 oat seeds on a black background under a F15T8-BLB or comparable ultraviolet tube(s) in an area where light from other sources is excluded. Seeds are considered fluorescent if the lemma or palea fluoresce or appear light in color. "Partially fluorescent" seeds shall be considered fluorescent. Seeds are considered nonfluorescent if the lemma and palea do not fluoresce and appear dark in color under the ultraviolet light.

BACKGROUND INFORMATION AND SUPPORTING EVIDENCE

Background information: The oat seed fluorescence test is listed as a cultivar testing procedure in the International Rules for Seed Testing (4).

This procedure was found useful for distinguishing between seeds of white (fluorescent) and yellow (nonfluorescent) oats when observed under an ultraviolet light (2). Finkner, et al., (3) evaluated seeds of 141 oat cultivars and selections for fluorescence in addition to investigating the inheritance of the fluorescence character. They classified cultivars as either fluorescent or nonfluorescent. Some variation in the intensity of fluorescence was observed among the cultivars classified as fluorescent. Although the shade of fluorescence was observed to vary for seed lots of some cultivars grown at different locations, there were no instances of a change from fluorescent or nonfluorescent categories. Finkner, et al., (3) reported

that fluorescence is dominant to nonfluorescence and is simply inherited. Two genes were reportedly involved in the expression of fluorescence (3). The presence of the dominant allele of one or both of the genes results in a seed being fluorescent (3). The involvement of two genes in oat seed fluorescence was substantiated by Sadanaga (7) and Chang and Sadanaga (1). Jones (5,6) observed numerous oat varieties under ultraviolet light and classified them as dark orange-bronze (nonfluorescent) or one of several shades of luminescence (fluorescent). Jones (6) also planted fluorescent and nonfluorescent oat seeds and found that plants grown from fluorescent seeds always produced fluorescent seeds and plants grown from nonfluorescent seeds always produced nonfluorescent seeds.

Supporting Evidence: A referee test conducted by the AOSA Cultivar Purity Subcommittee (1979-1980) involving 32 seed testing laboratories confirmed that the results of the oat seed fluorescence test are consistently reproducible by different laboratories. The testing procedure used successfully in the referee test is the same as the procedure listed in the proposed rule. This testing procedure is also included on pages 16, 17 and 18 of the Progress Report on the AOSA Cultivar Purity Testing Handbook published as Volume 62, No. 3 of the AOSA Newsletter in May 1988.

The oat seed fluorescence test is currently being used by a number of seed testing laboratories to help confirm that seed lots are correctly labeled as to variety and to help determine if certain seeds are contaminates. Therefore, this testing procedure should be incorporated into the AOSA Rules.

LITERATURE CITED

(1) Chang, T.D. and K. Sadanaga. 1964. Crosses of six monosomics in Avena sativa L. with varieties, species and chlorophyll mutants. Crop Sci. 4:589-593.

(2) Chmelar, F. and K. Mostovoj. 1938. On the application of some old and on the introduction of new methods for testing genuineness of variety in the laboratory. Proc. Int. Seed Test. Assoc. 10:68-74.

(3) Finkner, R.E., H.C. Murphy, R.E. Atkins, and D.W. West. 1954. Varietal reaction and inheritance of fluorescence in oats. Agron. J. 46:270-274.

(4) International Seed Testing Association. 1985. International rules for seed testing. Seed Sci. & Technol. 13:300-513.

(5) Jones, B. 1952. Treatments of Boone oats and observation under ultraviolet light. Proc. Off. Seed Anal. 42:80-82.

(6) Jones, B. 1953. Field plantings of some oat varieties observed under ultraviolet light. Proc. Assoc. Off. Seed Anal. 43:45-49.

(7) Sandanaga, K. 1970. Genetics and test of association of the fluorescence genes in hexaploid oats. Crop Sci. 10:103-104.

SUBMITTED BY

Richard C. Payne, Federal Seed Laboratory, USDA, AMS, Beltsville, MD. 20705

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3. PROPOSAL

Revised format for 3.2.b. in the Rules.

PRESENT RULE

3.2
b. Chemical tests. The phenol method for testing wheat (Triticum aestivum and other Triticum spp.) seed for cultivar purity is outlined in AOSA Handbook No. 28, "A Standardized Phenol Method for Testing Wheat Seed for Varietal Purity."

PROPOSED RULE

3.2.b. Chemical tests.
(1) Phenol Test for Wheat: The phenol method for testing wheat (Triticum aestivum and other Triticum spp.) seed for cultivar purity is outlined in AOSA Handbook No. 28, "A Standardized Phenol Method for Testing wheat Seed for Varietal Purity."

SUPPORTING EVIDENCE

If the first rule proposal (Peroxidase Test for Soybean) is adopted, then the editorial change suggested in this rule proposal should be made.

SUBMITTED BY

Richard C. Payne, Federal Seed Laboratory, USDA, AMS, Beltsville, MD. 20705

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4. PROPOSAL

Revision of APPENDIX 2 in the Rules, and the calculation of number of contaminant seeds in a sample.

PRESENT RULE

APPENDIX 2. CONVERSION OF SAMPLE WEIGHTS

Conversion Factors

Sample Weight (grams) Column I	Factor to be used for seed per pound Column II	Factor to be used for seed per ounce Column III
25	18.0	1.14
35	13.0	0.81
50	9.0	0.57
100	4.5	0.29
150	3.0	--
300	1.5	--
500	0.9	--

Directions and examples

1. To convert to a per pound basis the number of seeds found in a sample size listed in Column I, multiply by the number on the same line in Column II.

Example: In a 50-gram examination of alfalfa seed, 12 dodder seeds were found. 12 x 9 (Column II, line 3) = 108 dodder seeds per pound.

2. To convert to a per ounce basis, the number of seeds found in a sample size listed in Column 1, multiply by the number on the same line in Column III.

Example: In a 25-gram sample of bentgrass seed 7 oxeye daisy seeds were found. 7 x 1.14 (Column III, line 1) = 7.98 or 8 oxeye daisy seeds per ounce.

3. To convert the number of seeds per pound to any sample size shown in Column I, divide the number of seeds (such as that shown on a label) by the number in Column II on the line with the desired sample weight.

Example: Sorghum seed is labeled to show 12 Johnsongrass seeds per pound and the number found in a 300-gram examination is desired. 12 divided by 1.5 (Column II, line 6) = 8 Johnsongrass seeds in 300 grams.

4. To convert the number of seeds per ounce to any sample size shown in Column I, divide the number of seeds (such as that shown on a label) by the number in Column III on the line with the desired sample weight.

Example: White clover seed is labeled to show 8 seeds of curled dock per ounce and the number found in a 35-gram examination is desired. 8 divided by 0.81 (Column III, line 2) = 9.88 or approximately 10 curled dock seeds in 35 grams.

PROPOSED RULE

Change APPENDIX 2. CONVERSION OF SAMPLE WEIGHTS to read:

(A) When converting numbers of seeds found in the working sample to the number of seeds per pound, the following formula shall be used:

$$\frac{453.6 \text{ grams} \times \text{number of seeds found}}{\text{weight of working sample (grams)}} = \text{number of seeds per pound}$$

(B) When converting numbers of seeds found in the working sample to the number of seeds per ounce, the following formula shall be used:

$$\frac{28.35 \text{ grams} \times \text{number of seeds found}}{\text{weight of working sample (grams)}} = \text{number of seeds per ounce}$$

When using the above formulas, use the actual weight of the working sample (four significant figures). The final result shall be rounded to a whole number when reporting seeds per pound and to the first decimal place when reporting seeds per ounce. When rounding off the final result to a whole number, round down if the first decimal place is 4 or less and round up if the first decimal place is 5 or more. When rounding off the final result to the first decimal place, round down if the second decimal place is 4 or less and round up if the second decimal place is 5 or more.

Example 1. In a 50-gram noxious weed seed examination of alfalfa seed with an actual working weight of 50.13 grams, 7 dodder seeds were found. For number of seeds per pound (Formula A):

$$\frac{453.6 \text{ grams} \times 7}{50.15 \text{ grams}} = 63.31 \text{ seeds}$$

then

round to the nearest whole number

$$= 63 \text{ seeds per pound}$$

Example 2. In a 2 gram purity examination of white clover, with an actual working weight of 2.221 grams, 1 chickweed seed was found. For number of seeds per pound (Formula A):

$$\frac{453.6 \text{ grams} \times 1}{2.221 \text{ grams}} = 204.2 \text{ seeds}$$

then

round to the nearest whole number

= 204 seeds per pound

Example 3. In a 10 gram noxious weed seed examination of Kentucky bluegrass, with an actual working weight of 10.13 grams, 4 Canada thistle achenes were found. For number of seeds per ounce (Formula B):

$$\frac{28.35 \text{ grams} \times 4}{10.13 \text{ grams}} = 11.19 \text{ seeds}$$

then

round to one decimal place

= 11.2 seeds per ounce

Example 4. In a .25 gram purity examination of bentgrass, with an actual working weight of .2584 grams, 3 windgrass florets were found. For number of seeds per ounce (Formula B):

$$\frac{28.35 \text{ grams} \times 3}{.2584 \text{ grams}} = 329.14 \text{ seeds}$$

then

round to one decimal place

= 329.1 seeds per ounce

SUPPORTING EVIDENCE

The examples currently in APPENDIX 2 are outdated. The minimum working weights used in three of the examples far exceed the minimum working weights currently listed in Table 1. The table does not include many of the working sample weights covered in Table 1, particularly those below 25 grams. In addition the conversion factors used (450 gm/lb and 28.4 gm/oz) are not the actual conversions for grams to pounds or grams to ounces.

The conversion tables for determining rate of occurrence of noxious weed seed issued by the Federal government use the actual conversion factors for grams to pounds (453.59237 gm/lb) and grams to ounces (28.35 gm/oz). The final result is expressed as a whole number. These

tables are currently being used by the Federal government in the enforcement of the Federal Seed Act.

One purpose of the Rules For Testing Seeds is to standardize the methods used in seed testing. In speaking with numerous analysts it has become apparent that there is a lack of standardization in the methods used for converting grams to pounds and/or ounces. The conversion factor for grams to pounds varies from 453.59327 to 453.6 to 454 to 450. The conversion factor for grams to ounces varies from 28.35 to 28.4 to 28.

A second problem arises in whether to use the minimum working weight as shown in Table 1 or to use the actual working weight (to four significant figures) for the sample in question. In most cases the actual working weight is greater than the minimum working weight listed in Table 1. If the actual working weight is not used in the equation, the result would be an over-estimation of the number of contaminants per pound or per ounce, except in those cases when the working weight is exactly the minimum required. If Example 2 (white clover purity) in the proposal were to be calculated using the whole number minimum working weight of 2 grams, the number of chickweed seeds per pound would be 227 rather than the 204 seeds per pound found when using the actual working weight.

Expression of the final answer as a whole number or as a fraction represents a third problem. Most laboratories round off the final answer to the nearest whole number by rounding up if the number following the decimal is 5 or larger and by rounding down if the number is less than 5. The problem of rounding off to the nearest whole number becomes evident in some states where regulations provide for labeling the number of contaminants either per pound or per ounce. If, for example, 7 quackgrass seeds were found in a 500 gram noxious weed seed examination of oat seed, the rate per pound would be 6 per pound (as recommended in the proposal), or the rate per ounce would be 0.4 per ounce (as recommended in the proposal). If the results were rounded to the nearest whole number, then this sample could be labeled 0 quackgrass per ounce.

Rather than develop a lengthy "table" format and attempt to cover all possible working weights and numbers of seeds found, two simple formulas are proposed; one based on number of contaminants found per pound and one based on number of contaminants found per ounce. With this method analysts need not refer to a chart each time a

contaminant needs to be reported. The formulas are simple to use, easy to memorize and far more accurate since they incorporate the use of the actual working weight of the sample.

SUBMITTED BY

Deborah J. L. Meyer, Uniformity Subcommittee
California Dept. of Food & Agriculture
Analysis & Identification/Seed Laboratory
1220 N Street, Room 340
Sacramento, California 94271-0001

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5. PROPOSAL

Change seed unit in section 2.6 b. for Festuca spp. (excluding Festuca rubra) and Lolium spp. from caryopses and single florets to multiple florets (as well as caryopses and single florets).

PRESENT RULE

2.6 b. (2)	Multiple florets and spikelets in tall oatgrass (Arrhenatherum elatius), oat (Avena spp.), gramas (Bouteloua spp.), rhodesgrass (Chloris gayana), barley (Hordeum vulgare), and bluegrass (Poa spp.);

PROPOSED RULE

2.6 b.(2)(a)	Multiple florets in fescue (Festuca spp. excluding red and creeping red fescue, Festuca rubra subsp. rubra; and chewings fescue, Festuca rubra subsp. commutata), and ryegrass (Lolium spp.);
2.6 b.(2)(b)	Multiple florets and spikelets in tall oatgrass (Arrhenatherum elatius), oat (Avena spp.), gramas (Bouteloua spp.), rhodesgrass (Chloris gayana), barley (Hordeum vulgare), and bluegrass (Poa spp.);

SUPPORTING EVIDENCE

According to the present rules, only caryopses and single florets are considered seed units for Festuca spp. (excluding Festuca rubra subsp. rubra, and Festuca rubra subsp. commutata), and Lolium spp. This ruling makes it mandatory that any attached sterile and fertile florets be removed. Seed units are the structures

usually regarded as a seed in planting practices and in commercial channels. This statement in the Rules certainly makes multiple florets of the above mentioned species the seed unit. Last year A.O.S.A. moved Lolium spp. from the nonchaffy seed purity tolerance section to the chaffy seed section, placing them with other grasses that have multiple structured seed units.

In 1959 Dan Niffenegger at Montana State University could not find a satisfactory modified method for the testing of Lolium spp. and Festuca arundinacea, although modified methods were found for ten other chaffy grasses.

The 1980-1983 International Seed Testing Association Purity Committee concluded that the percentage of inert matter varies so much in Lolium spp. that use of a conversion factor was out of the question. I.S.T.A. now considers a sterile floret attached to a fertile floret the seed unit, provided that the sterile floret does not extend to the tip of the fertile floret. Multiple seed units of Festuca spp. are left intact and are included in the pure seed fraction. Purity results of the seed lots included in the survey showed only a slight increase in percentage pure seed using the new method. Germination tests of seed lots included in their survey showed no significant difference in the percent normal seedlings for multiple florets versus single florets.

If adopted, this proposal will move the AOSA testing procedures for these kinds in a direction that more closely relates to I.S.T.A. testing procedures, and will eliminate the time consuming job of removing attached florets.

Copies of the research data and published papers submitted with this proposal are available from the chairman of the Rules Committee upon request.

SUBMITTED BY: Jim Effenberger, Uniformity Subcommittee
California Dept. of Food & Agriculture
Analysis & Identification/Seed Laboratory
1220 N Street, Room 340
Sacramento, California 94271-0001

6. PROPOSAL

Addition of TB as substratum and 10°C as temperature and a change in Additional Directions for tidy tips-daisy in Table 4.

PRESENT RULE

Kind of Seed	Substrata	Temp. °C.	First count days	Final count days	Additional Directions
Layia platyglossa tidy tips-daisy	P	15	4	8	Light; new crop seed very sensitive to warm temperatures.

PROPOSED RULE

Kind of Seed	Substrata	Temp. °C.	First count days	Final count days	Additional Directions
Layia platyglossa tidy tips-daisy	P,TB	10,15	4	8	Light. New crop seed may require 10°C (dark) for rapid, maximum response.

SUPPORTING EVIDENCE

When six samples were tested in our lab at 10°C. and 15°C., their respective germination means were 82% and 50%.

Sample	10°C.,dark	15°C.,light
1	77	37
2	62	62
3	93	72
4	84	42
5	86	60
6	87	28

SUBMITTED BY

Ellen Chirco
N.Y. Seed Lab., NYSAES
Geneva, New York 14456

7. PROPOSAL

Addition of Rudbeckia hirta - black-eyed Susan to the Rules.

PROPOSED RULE

Include in Table 4 (Methods of testing for laboratory germination, FLOWER SEEDS) the following:

Kind of Seed	Substrata	Temp. °C.	First count days	Final count days	Additional Directions
Rudbeckia hirta L. black-eyed Susan	TB	20-30	7	14	Light.

SUPPORTING EVIDENCE

1) Sluis en Groot's "Methods of Artificial Seed Testing" suggests 20-30°C. for 14 days.

2) On 13 samples tested of Rudbeckia hirta, our lab had results as shown in the following table:

% Germination of Rudbeckia hirta

Sample	20°C. Light	20-30°C. Light	7 day prechill 20-30°C. Light	7 day prechill and KNO_3, 20-30°C. Light
1	3	34	23	46
2	82	80	85	88
3	80	79	83	84
4	75	90	90	89
5	76	78	75	83
6	69	72	63	70
7	77	76	59	70
8	75	79	74	72
9	70	71	62	64
10	73	78	75	69
11	63	68	62	52
12	92	94	92	93
13	65	65	60	48
Mean:	69%	74%	70%	71%

SUBMITTED BY

Ellen M. Chirco
NY State Seed Lab., NYSAES
Geneva, New York 14456

8. PROPOSAL

Addition of Ratibida columnifera - Mexican hat, prairie coneflower to the Rules.

PROPOSED RULE

Include in Table 4 (Methods of testing for laboratory germination, FLOWER SEEDS) the following:

Kind of Seed	Substrata	Temp. °C.	First count days	Final count days	Additional Directions
Ratibida columnifera (Nuttall) Wooton & Standley Mexican hat, prairie coneflower	TB	15,20	7	14	Light.

SUPPORTING EVIDENCE

Eleven samples were tested in our lab at 15°C., 20°C., and 20-30°C. with light, with and without KNO_3 and with a 7 day prechill (then 20-30°C.). Since KNO_3 and the prechill did not enhance germination, results under these conditions are not shown. Test results and mean germination percentages are given below.

% Germination of Ratibida columnifera

Sample	15°C. Light	20°C. Light	20-30°C. Light
1	68	75	53
2	69	63	42
3	90	95	89
4	84	85	88
5	61	67	69
6	75	79	74
7	87	92	85
8	67	73	58
9	67	75	65
10	57	51	33
11	50	64	52
Mean	71%	75%	64%

SUBMITTED BY

Ellen M. Chirco
N.Y. Seed Lab., NYSAES
Geneva, New York 14456

9. PROPOSAL

Addition of Monarda citriodora - lemon mint to the Rules.

PROPOSED RULE

Include in Table 4 (Methods of testing for laboratory germination, FLOWER SEEDS) the following:

Kind of Seed	Substrata	Temp. °C.	First count days	Final count days	Additional Directions
Monarda citriodora Lagasca lemon mint	TB	15,20, 15-25	7	21	Light.

SUPPORTING EVIDENCE

Nine samples were tested in our laboratory at 15°C., 20°C., 15-25°C. and 20-30°C., with light, with and without KNO_3, and with a 7 day prechill (then 20-30°C.). Since KNO_3 and prechill did not enhance germination, results under these conditions are not shown. Other test results and mean germination percentages are given in the following table:

% Germination of Monarda citriodora

Sample	15°C. Light	20°C. Light	20-30°C. Light	15-25°C. Light
1	81	83	68	89
2	78	70	81	74
3	73	74	65	72
4	75	75	65	67
5	51	53	43	47
6	48	40	40	53
7	67	47	32	38
8	61	69	56	54
9	89	78	82	75
Mean	69%	65%	59%	63%

SUBMITTED BY.

Ellen M. Chirco
N.Y. Seed Lab., NYSAES
Geneva, New York 14456

10. PROPOSAL

Addition of Cowania mexicana - cliffrose to the Rules.

PROPOSED RULE

1) Include in Table 1 (Weights for working samples, TREE and SHRUB SEEDS) the following:

Kind of Seed	Min. Wt. for Purity Anal.(g)	Approx. No. Seeds/Gram	Approx.No. Seeds/Oz.
Cowania mexicana D. Don cliffrose	19	130	3650

2) Include in Table 5 (Methods of testing for laboratory germination, TREE and SHRUB SEEDS) the following:

Kind of Seed	Substrata	Temp.°C	Dur.	Add. Dir.
Cowania mexicana cliffrose	B,P	15,10-30	28	Prechill 30 days at 1-2°C.; or use TZ[b].

SUPPORTING EVIDENCE OR REASONS FOR THE PROPOSED RULE

1) Seed weights are based on 6 lots and are in approximate agreement with values reported elsewhere (Alexander et al 1974; Belcher 1985). The seed unit is an indehiscent one-seeded fruit. The plumose style is removed in the commercial cleaning process. Lots are usually cleaned to high purity, and purity analysis is not a problem.

2) Cliffrose seeds are largely dormant at harvest and require moist prechill in order to germinate. Experiments to determine the minimum chill period required were conducted on 1987 and 1988 seedlots. In the 3-lot 1987 experiment, germination percentages were as follows: 4-week chill--80%, 6-week chill--85%, 8-week chill--89%, TZ viability--89%. Chill was carried out at 1°C. In the 3-lot 1988 experiment, the 4-week chill was carried out at 2 temperatures: 1°C germination--42%, 3-5°C germination--32%, TZ viability--54%. These results suggest that a 4-week chill is adequate for germination of 80-90% of viable seeds as long as the chill temperature is low. Increasing the chill to 6 weeks results in germination of 95-100% of viable seeds.

Most workers have reported that a relatively short chill is adequate for cliffrose (Alexander et al 1974; Young and Evans 1981; Belcher 1985). Adequately chilled seed is not temperature sensitive, but marginally chilled

seed may respond to light and/or alternating regimes (Heit 1970, Young and Evans 1981).
3) Viability evaluation using TZ is straightforward for this species. Mean maximum germination (6-8 week prechill) was identical to total viable seed percentage as determined by TZ on a separate sample for both experiments described above.
4) In a referee germination test involving 7 laboratories, mean germination percentage was 44.4 (S.E=6.1%, range 24-63%).
The variable results probably reflect the effect of conditions during chilling. Total viable seed percentages were much less variable (mean=81%, S.E.=1.7%, range 74-86%), indicating that the analysts were able to TZ ungerminated seeds consistently at the end of the test.

Our recommendation is a 30 day prechill for the germination test at 1-2°C. The percentage gained in a longer chill does not seem worth the additional time, especially since TZ on ungerminated seeds is relatively easy and accurate. We recommend TZ as an alternate method for viability determination.

More detailed supporting evidence was received by the Rules Committee and is available on request.

LITERATURE CITED

Alexander, R. R., K. R. Jorgensen, and A. P Plummer. 1974. Cowania. p. 353-355. In: Schopmeyer, C. S.(ed.). Seeds of woody plants in the United States. USDA Handbook 450.

Belcher, E. (ed.). 1985. Handbook on seeds of browse-shrubs and forbs. USDA Forest Service Tech. Pub. R8-TP8.

Heit, C. E. 1970. Germinative characteristics and optimum testing methods for twelve western shrub species. Proc. AOSA 60:197-205.

Young, J. A., and R. A. Evans. 1981. Germination of seeds of antelope bitterbrush, desert bitterbrush, and cliffrose. USDA Science and Education Administration ARR-W17/January 1981.

SUBMITTED BY

Susan E. Meyer[1], Stanley L. Kitchen[1], G. Richard Wilson[2], and Richard Stevens[3]

1 USDA Forest Service Intermountain Research Station
2 Utah State Seed Laboratory
3 Utah Division of Wildlife Resources

11. Proposal

Addition of Cercocarpus ledifolius - curlleaf mountain-mahogany to the Rules.

PROPOSED RULE

1) Include in Table 1 (Weights for working samples, TREE and SHRUB SEEDS) the following:

Kind of Seed	Min. Wt. for Purity Anal.(g)	Approx. No. Seeds/Gram	Approx.No. Seeds/Oz.
Cercocarpus ledifolius Torrey & A. Gray curlleaf mountain-mahogany	25	100	3000

2) Include in Table 5 (Methods of testing for laboratory germination, TREE and SHRUB SEEDS) the following:

Kind of Seed	Substrata	Temp.°C	Dur.	Add. Dir.
Cercocarpus ledifolius curlleaf mountain-mahogany	B,P	15,10-30	28	Prechill 70 days at 1-2°C.

SUPPORTING EVIDENCE OR REASONS FOR THE PROPOSED RULE

1) Seed weights are based on 8 lots and are in rough agreement with values reported elsewhere (Deitschman et al 1974, Belcher 1985). The seed unit is an indehiscent one-seeded fruit. The plumose style is removed in the commercial cleaning process. Lots are usually cleaned to high purity, and purity analysis is not a problem.

2) Curlleaf mountain-mahogany seeds are largely dormant at harvest and require a moist prechill in order to germinate. Efforts to circumvent the chill requirement by soaking with water, hydrogen peroxide, gibberellic acid, and by clipping did not produce consistently satisfactory results.

Experiments were carried out in order to determine the minimum chill required to break dormancy. In 1986, all 5 lots germinated to a value not significantly different from the maximum after a 12-week chill; an 8-week chill was adequate for 3 of 5 lots. In an experiment with 6 lots of 1987 seed, mean germination percentages were: 8-week chill--84%, 10-week chill--87%, 12-week chill--89%, 16-week chill--90%.

Previous workers have reported varying results in chill experiments with this species but 1-2°C is proposed

as the optimum. Young et al (1978) got no response to chilling at 2°C and 5°C for periods of up to 12 weeks. Chilling in aerated water under the same regimes gave full germination, suggesting that aeration (i.e. degree of moisture in the substratum) is critical. Liacos and Nord (1961) reported that 30-90 days of prechill was effective, while Heit (1970) reported no increase in germination when the chill period was increased from 30 to 90 days. Treatment with sulfuric acid prior to chilling has sometimes been recommended but does not seem a suitable approach for routine use because of the danger of damaging some lots (Heit 1970, Belcher 1985).

The seed is not temperature sensitive once it has been adequately chilled (Young et al 1978), but marginally chilled seeds may respond to alternating regimes (Heit 1970).

3) Results of a 6-laboratory referee germination test reflect problems associated with chill conditions and post-test viability evaluation. Dormant seed values ranged from 0-61%; total viable seed values were less variable, with 5 of 6 values ranging from 74-90%. Laboratories which handle this seed on a regular basis obtained total viable seed percentages which were consistent with each other. Experience is undeniably important in accurately testing this seed.

4) Results of a referee tetrazolium test confirmed the acknowledged difficulty with TZ procedures for this seed (Belcher 1985). Values ranged from 56-97%. We are unable to recommend TZ as a alternative procedure for determining viability.

More detailed supporting evidence was reviewed by the Rules Committee and is available on request.

LITERATURE CITED

Belcher, E. (ed.) 1985. Handbook on seeds of browse-shrubs and forbs. USDA Forest Service Tech. Pub. R8-TP8.

Deitschman, G. H., K. R. Jorgensen, and A. P. Plummer. 1974. Cercocarpus. In: Schopmeyer, C. S. (ed.). Seeds of woody plants in the United States. USDA Handbook 450.

Heit, C. E. 1970. Germinative characteristics and optimum testing methods for twelve western shrub species. Proc. AOSA 60:197-205.

Liacos, L. G., and E. C. Nord. 1961. Curlleaf cercocarpus dormancy yields to acid and thiourea. J. Range Manage. 14:317-320.

Young, J. A., R. A. Evans, and D. L. Neal. 1978. Treatment of curlleaf cercocarpus seeds to enhance germination. J. Wildl. Manage. 42:614-620.

SUBMITTED BY

Stanley L. Kitchen[1], Susan E. Meyer[1], G. Richard Wilson[2], and Richard Stevens[3]

1 USDA Forest Service Intermountain Research Station
2 Utah State Seed Laboratory
3 Utah Department of Wildlife Resources

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12. Proposal

Addition of Cercocarpus montanus - true mountain-mahogany to the Rules.

PROPOSED RULE

1) Include in Table 1 (Weights for working samples, TREE and SHRUB SEEDS) the following:

Kind of Seed	Min. Wt. for Purity Anal.(g)	Approx. No. Seeds/Gram	Approx.No. Seeds/Oz.
Cercocarpus montanus Rafinesque true mountain-mahogany	28	90	2500

2) Include in Table 5 (Methods of testing for laboratory germination, TREE and SHRUB SEEDS) the following:

Kind of Seed	Substrata	Temp.°C	Dur.	Add. Dir.
Cercocarpus montanus true mountain-mahogany	B,P	15,10-30	28	Prechill 60 days at 1-2°C; or use TZ[b].

SUPPORTING EVIDENCE OR REASONS FOR THE PROPOSED RULE

1) Seed weights are based on 10 lots and are in rough agreement with values reported elsewhere (Deitschman et al 1974, Belcher 1985). The seed unit is an indehiscent one-seeded fruit. The plumose style is removed in the commercial cleaning process. Lots are usually cleaned to high purity, and purity analysis is not a problem.
2) True mountain-mahogany seeds are largely dormant at harvest and require moist prechill in order to germinate. Efforts to circumvent the chill requirement by soaking with water, hydrogen peroxide, or gibberellic acid and by clipping were not consistently satisfactory.

Experiments to determine the minimum chill period required were conducted on 6 lots in 1986 and on 9 lots in 1987. In the 1986 experiment, 5 of 6 lots had germination percentages not significantly different from the maximum after 8 weeks of chill. Mean germination after 8 weeks of chill was the same as mean total viable seed percentage as estimated using TZ. Similar results were obtained in the 1987 experiment. Mean germination was 79% after an 8-week chill and 86% after a 12-week chill; mean total viable seed percentage as determined by TZ on separate samples was 86%.

Other workers have reported that a chill of 4 to 9 weeks effectively breaks dormancy (Heit 1970, Deitschman et al 1974, Piatt 1976, Belcher 1985). Temperatures of 5°C and over are apparently not effective (Moore 1963) and 1-2°C is proposed as the optimum. Adequately chilled seed is not temperature sensitive, but marginally chilled seed germinates better at alternating regimes (Heit 1970, Piatt 1976).

3) Results of a 7-laboratory germination referee using the proposed rule were somewhat uneven. Germination percentages varied from 44-70%, while total viable seed percentages were less variable (60-79%). An alternate rule proposal involving clipping in combination with a 4-week chill resulted in reduced total viable seed percentages and was not considered further. Variation in referee results is probably due to variations in efficacy of the chilling environment and to technical problems in determination of viability of ungerminated seed. Tetrazolium staining in this species is difficult and is recommended as an alternative to the lengthy germination test with considerable reservation (Belcher 1985).

More detailed supporting evidence was received by the Rules Committee and is available on request.

LITERATURE CITED

Belcher, E. (ed.). 1985. Handbook on seeds of browse-shrubs and forbs. USDA Forest Service Tech. Pub. R8-TP8.

Deitschman, G. H., K. R. Jorgensen, and A. P. Plummer. 1974. Cercocarpus. p. 309-312. In: Schopmeyer, C. S. (ed.). Seeds of woody plants in the United States. USDA Handbook 450.

Heit, C. E. 1970. Germinative characteristics and optimum testing methods for twelve western shrub species. Proc. AOSA 60:197-205.

Moore. T. C. 1963. A germination inhibitor in achenes of Cercocarpus montanus. Ecology 44:406-409.

Piatt, J. R. 1976. Effects of water stress and temperature on germination of true mountain-mahogany. J. Range Manage. 29:138-140.

SUBMITTED BY

Stanley L. Kitchen[1], Susan E. Meyer[1], G. Richard Wilson[2], and Richard Stevens[3].

1 USDA Forest Service Intermountain Research Station
2 Utah State Seed Laboratory
3 Utah Division of Wildlife Resources

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13. PROPOSAL

Addition of Chrysothamnus nauseosus - rubber rabbitbrush to the Rules.

PROPOSED RULE

1) Include in Table 1 (Weights for working samples, TREE and SHRUB SEEDS) the following:

Kind of Seed	Min. Wt. for Purity Anal.(g)	Approx. No. Seeds/Gram	Approx.No. Seeds/Oz.
Chrysothamnus nauseosus (Pursh) Britton rubber rabbitbrush	2	1350	38,200

2) Include in Table 5 (Methods of testing for laboratory germination, TREE and SHRUB SEEDS) the following:

Kind of Seed	Substrata	Temp.°C	Dur.	Add. Dir.
Chrysothamnus nauseosus rubber rabbitbrush	P	25,20-30	28	

SUPPORTING EVIDENCE OR REASONS FOR THE PROPOSED RULE

1) Seed weights are based on filled fruits of 16 samples belonging to subspecies commonly encountered in commerce. The pappus may or may not be removed in cleaning; if attached it was considered part of the seed unit. Seed weights in this species are variable. The values here are in rough agreement with values reported elsewhere (Deitschman et al 1974, Belcher 1985).

2) In a referee purity analysis, laboratories were asked to include only filled fruits as pure seed. Purity percentages were in close agreement (mean 43.8, S.E. 0.6, range 41.7-46.1, n=8). These results suggest that a pure seed criterion similar to that used for weed seed (2.10B

(4)) would work well for this species, which is often sold at low purity. Results of purity analyses on 6 commercial lots indicated that decisions regarding inclusion or exclusion of unfilled fruits had no major effect on pure live seed values (mean 15.0% versus 18.8% respectively). We recommend following the present rule for pure seed (2.7 f.) rather than proposing a global change in purity analysis procedures for ASTERACEAE or a specific exception for rubber rabbitbrush.

3) Results of extensive germination experiments with over 60 collections of native rubber rabbitbrush seed indicate that dormancy at temperatures above 20°C is rare (Meyer and McArthur 1987). Germination of most lots likely to be encountered in commerce is complete within 14 days at 25°C, but some high elevation lots may require up to 28 days. Dormancy at intermediate temperatures (i.e. 15°C) is common in middle and high elevation collections; therefore relatively high temperature regimes are recommended (Meyer, McArthur, and Jorgensen in review). Lack of dormancy at high temperature has been reported by other workers (Sabo et al 1979, Belcher 1985, Romo and Eddleman 1988).

4) A germination referee using the proposed rule gave variable results (total viable seed percentage mean 63.8, S.E. 5.3, range 36.0-79.2, n=7). This may have been due to the large number of broken seeds in the sample, resulting in variable classification of abnormal seedlings.

A manuscript draft and more detailed supporting evidence was received by the Rules Committee and is available on request.

LITERATURE CITED

Belcher, E. (ed.) 1985. Handbook on seeds of browse-shrubs and forbs. USDA Forest Service Tech. Pub. R8-TP8.

Deitschman, G. H., K. R. Jorgensen, and A. P. Plummer. 1974. Chrysothamnus. p.326-328. In: Schopmeyer, C. S. (ed.) Seeds of woody plants in the United States. USDA Handbook 450.

Meyer, S. E., and E. D. McArthur. 1987. Studies on the seed germination biology of rubber rabbitbrush. p. 19-25. In: K. L. Johnson (ed.). Proc. Fourth Utah Shrub Ecology Workshop. The Genus Chrysothamnus. Utah State University, Logan, Utah.

Meyer, S., E. D. McArthur, and G. L. Jorgensen. In review. Variation in germination response to temperature in rubber rabbitbrush (Chrysothamnus nauseosus, Asteraceae) and its ecological significance.

Romo, J. T., and L. E. Eddleman. 1988. Germination of green and gray rubber rabbitbrush and their establishment on coal mined land. J. Range Manage. 41:491-495.

Sabo, D. G., G. V. Johnson, W. C. Martin, and E. F. Aldon. 1979. Germination of 19 species of arid land plants. USDA Forest Service Res. Pap. RM-210.

SUBMITTED BY

Susan E. Meyer[1], G. Richard Wilson[2], and Richard Stevens[3]

1 U.S.Forest Service Intermountain Research Station
2 Utah State Seed Laboratory
3 Utah Division of Wildlife Resources