RULE PROPOSALS - 1994

AOSA Rules Committee
Jim Effenberger, Chair

The following proposals for changes in or additions to the AOSA Rules For Testing Seeds have been reviewed and approved by the Rules Committee. Approval does not mean that the committee or the members endorse these changes or additions to the Rules, only that the proposals meet the requirements which allows them to be addressed at the 1994 AOSA\SCST Portland, Oregon Meeting.

Seven proposals are presented here as required by the AOSA Constitution in order that the membership can review and evaluate them 90 days in advance of the AOSA business meeting. Please read and review all proposals and the supporting evidence carefully.

Comments concerning these proposals can be forwarded to the Rules Committee Chair. Your comments will be presented and discussed at the Open Rules Committee meeting in June. The names and addresses of the authors are noted if you wish to contact them for additional information. Extensive changes to these proposals are NOT possible at the Open Rules meeting.

Only a limited number of copies of these proposals will be available at the Open Rules meeting. We recommend that you bring your copy of this Newsletter with you to the AOSA\SCST meeting.

PROPOSAL #1

RULE PROPOSAL

PRESENT RULE (If new rule, state "New Rule")

2.1 Working sample
Purity. —— The sample on which the purity analysis is made. Noxious-weed seed. ——— The sample on which the noxious-weed seed examination is made.

PROPOSED RULE (Exactly as it would appear in "Rules")

2.1 Definitions

a. Coated/Pelleted seed: The deposition of a layer of inert materials that obscure the original shape and size of the seed resulting in a substantial weight increase and improved plantability. The addition of biologicals, pesticides, identifying colorants or dyes and/or other active ingredients including polymers can be included in this process.

b. Film-coated seed: Film-coated seed retains the shape and the general size of the raw seed with a minimal weight gain. The film coating may contain polymers, pesticides, biologicals, identifying colorants or dyes, and other additives. The coating should result in a more or less continuous covering which eliminates or minimizes product dust-off.

c. Inoculated seed: Seed which has received a coating of a commercial preparation containing a microbial product e.g. Rhizobium sp.

d. Primed seed: Seed that has been subjected to a procedure (biotic or abiotic) that reduces dormancy, promotes faster and/or more uniform germination.[1]

e. Raw seed: Seed that is free of any added foreign or inert materials. Not inoculated, treated, pelleted or coated.

f. Treated seed: Seed with a minimal covering of various materials whose primary objective is to reduce or control certain disease organisms, insects or other pests attacking the seed or seedlings growing therefrom and contains identifying colorants or dyes.

g. Working Sample: Purity. —— The sample on which the purity analysis is made. Noxious-weed seed. —— The sample on which the noxious-weed seed examination is made.

[1] Not to include physical processes such as scarification which are normally considered a conditioning operation.

SUPPORTING EVIDENCE (Research data, literature citations, published papers, or other appropriate information)

Rules Change Documentation:

The types of seed in commerce continues to change and some confusion exists as to the proper method of analysis for specific seed types. The attached recommendations represent the efforts of a large number of individuals and groups over a more than two year period. The original Definition Subcommittee was a subcommittee of the ASTA Seed Coating Subcommittee (Stu Barclay, chair) and J. S. Burris was appointed as chair of that Sub-Subcommittee in November of 1991. The initial committee was composed of: Larry Nees, Chip Sundstrom, Deborah Meyer, Doug Lawrence, Jeff Boris , Kyle Rushing, Peter Peerbolte, Stu Barclay, John Walsh, Alan Galbreth and Joe Burris (chair). A request for definitions of seed types was mailed to the entire membership (54 members) of the Seed Coating Subcommittee. The responses from that mailing were summarized and resubmitted to the members of the definition committee. The results of that process resulted in the suggested definitions which were presented to the Pacific Seedsmen Association meeting May of 1992. The revised definitions were then presented to the AOSA at the Oklahoma City meeting in June of 1992. Further responses were obtained and revisions were made by the committee and the updated definitions were presented as part of a presentation to the ASTA Corn Sorghum Research Conference in December of 1992 and to the Independent Professional Seedsmen Association Meeting, January 1993. The proposed changes were then formalized and the AOSA Board was asked for their endorsement at the Fort Collins meeting in June of 1993. Further discussion and revision have followed that meeting and the attached Rules change represents a synthesis of the best of the recommendations received during this two year period. It clearly defines the seed products currently in commerce. The inclusion of these as definitions near the front of the Purity section complements the Germination Definitions (4.2) and will provide a clear explanation of the seed types and how they should be tested.

SUBMITTED BY (Name, complete address, and phone number)

For further specifics please contact Dr. J. S. Burris, Seed Science Center, Iowa State University, Ames, Iowa 50011. phone 515-294-1880 or fax 515-294-2014.

DATE OF PROPOSAL 9/27/93

RULE PROPOSAL

PRESENT RULE (If new rule, state "New Rule")

2.13 Coated seed purity procedures

a. Definition: coated seed is a seed unit covered with any substance which changes the size, shape, or weigh of the original seed. Seeds coated with ingredients such as, but not limited to, rhizobia, dyes, and pesticides are excluded.

4.8 Special procedures and alternate methods for germination

1. Coated Seed:

PROPOSED RULE (Exactly as it would appear in "Rules")

2.13 Coated\Pelleted seed purity procedures

a. Where reference is made to coated seed the rules also apply to pelleted seed.

4.8 Special procedures and alternate methods for germination

1. Coated Seed: Where reference is made to coated seed the rules also apply to pelleted seed.

SUPPORTING EVIDENCE (Research data, literature citations, published papers, or other appropriate information)

If section 2.1 is changed as suggested by Dr. Burris, then sections 2.13 and 4.8 must also be changed.

SUBMITTED BY (Name, complete address, and phone number)

Dr. Joe Burris, Iowa State University, Seed Science Center, Ames IA 50011
phone: (515) 294-1880

DATE OF PROPOSAL 9/27/93

PROPOSAL #2

PRESENT RULE (If new rule, state "New Rule")

4.9.a
Substrata. - Symbols for substrata in column 2, Tables 3 and 4 are: B = between blotters; TB = top of blotters; T = paper toweling, used either as folded towel tests or as roll towel tests in horizontal or vertical position; S = sand or soil; TS = top of sand or soil; P = covered petri dishes with (a) two layers of blotters, or (b) three thicknesses of filter paper, or (c) top of sand or soil; C = creped cellulose paper wadding (0.3-inch thick Kimpak or equivalent) covered with a single thickness of blotter through which holes are punched for the seed which are pressed for about one-half their thickness into the paper wadding; RB = blotters with raised covers, prepared by folding up the edges of the blotter to form a good support for the upper fold which serves as a cover, preventing the top from making direct contact with the seeds; TC = on top of creped cellulose paper without a blotter.

PROPOSED RULE

4.9.a
Substrata. - Symbols for substrata in column 2, Tables 3 and 4 are: B = between blotters; TB = top of blotters; T = paper toweling, used either as folded towel tests or as roll towel tests in horizontal or vertical position; S = sand; TS = top of sand; P = covered petri dishes with (a) two layers of blotters, or (b) three thicknesses of filter paper, or (c) top of sand; C = creped cellulose paper wadding (0.3-inch thick Kimpak or equivalent) covered with a single thickness of blotter through which holes are punched for the seed which are pressed for about one-half their thickness into the paper wadding; RB = blotters with raised covers, prepared by folding up the edges of the blotter to form a good support for the upper fold which serves as a cover, preventing the top from making direct contact with the seeds; TC = on top of creped cellulose paper without a blotter.

Since it is generally difficult to obtain consistent supplies of soil or artificial compost, it is not recommended as primary testing substrate. However, it may be necessary to use it, for example, when seedlings show phytotoxic symptoms or if evaluation of seedlings is in doubt on paper. Soil is commonly used for comparative or investigative purposes. Refer to section 4.5b(1).

SUPPORTING EVIDENCE OF REASONS FOR THE RULE (Summarize reasons for the proposal. Supporting evidence should include nine copies of research data, literature citations, copies of published papers or any other information which would help the Committee during review.)

This rules proposal is submitted by the AOSA working group of the AOSA/ISTA Harmonization Committee. It addresses concerns on the part of the EEC representatives about variation included in germination testing as a result of the use of a substrate which may not be standardizable. It is submitted for the consideration of the AOSA membership.

SUBMITTED BY: (Name, Address, and Phone Number)

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DATE: October 13, 1993

Rules Proposal

Kind of seed: All kinds.

Present Rule:

4.5.b. Guides for evaluation of seedlings.

(2) Photographs of normal and abnormal seedlings. -- These were prepared by the Federal Laboratory, Beltsville, Maryland, and approved by the Association. The negative numbers are listed in Table 3 under the column heading "Specific requirements and photograph numbers."

4.9 Explanation of Tables 3, 4 and 5

j. Photographs of seedlings -- The photograph numbers listed in the column "Additional Directions" identify U.S. Department of Agriculture photographs of some types of normal and abnormal seedlings. [a]

Page 21, footnote a

Only the photographs described in the AOSA Newsletter 57(3):67-72 (September 1983) may be purchased from the Office of Information, United States Department of Agriculture, Washington, D.C. 20250.

4.10, Table 3. Methods of testing for laboratory germination, AGRICULTURAL SEEDS

Heading to Column 6 -- Specific requirements and photograph numbers (See Sec. 4.9-b-e-f-j)

References to photos in column 6 throughout Table 3, e.g. page 53, *Arachis hypogaea*: Remove shells; photos 19541, 19542.

Proposed Rule:

Delete the following sections:

4.5.b. (2)

4.9.j.

Delete footnote (a), page 21.

Change wording in heading to Column 6 of Table 3 to:

"Specific requirements (See Sec. 4.9-b-e-f)"

Delete references to photos in column 6 throughout Table 3,

e.g. page 53, *Arachis hypogaea*: Remove shells. ~~photos 19541, 19542.~~

Reasons for the proposed change:

When the Seedling Evaluation Handbook was developed, it was the intention of the Committee that the Handbook would serve to replace the photographs referenced in the Rules. The Handbook was adopted through a series of rules change proposals in 1989 and 1991. These proposals should have included one to delete the references to the photos, but this was overlooked.

With the adoption of the Seedling Evaluation Handbook, the drawings became the standard for seedling evaluation under the AOSA Rules. As long as the references to the photographs remain in the Rules there are two sets of standards in effect, which creates a real potential for unresolvable conflicts in evaluation. Since the drawings were adopted as the standard by the Association in 1991, all references to the photographs must be deleted so that only one standard exists.

Submitted by: D. Ashton
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October 1, 1993

PROPOSAL #4

AOSA RULES COMMITTEE

PROPOSAL:

Addition of T as substratum for Spinach, Spinacia oleracea, germination in Table 3.

PRESENT RULE:

Kind of Seed	Substrate	Temp. Cent.	First Count Days	Final Count Days
Spinacia oleracea spinach	TB	15; 10	7	21

PROPOSED RULE:

Kind of Seed	Substrate	Temp. Cent.	First Count Days	Final Count Days
Spinacia oleracea spinach	TB, T	15; 10	7	21

SUPPORTING EVIDENCE:

The AOSA Region I, 1992-1993, Spinach germination referee on blotter versus rolled towel supported the use of rolled towel as a substrate. The rolled towel and blotter germinations obtained similar germinations across 21 labs on 3 commercial lots. The range of germination percentages between labs was reduced with rolled towel from 1 to 7% compared to blotter germinations between labs, Tables 1, 2, and 3.

The analysis of variance given in the referee was reviewed and revised by Dr. Dan Niffenegger, University of Kentucky. Both the original analysis of variance and the revised analysis of variance found that the only treatment not of significant at the 1% level of probability was the treatment of substrate. Treatments found to be significant were samples (sa), labs (L), interactions of L x Sa and L x Substrate and error B. The statistical analysis design was based on a 2 x 3 x 4 x 21 factorial with 503 degrees of freedom, Table 4.

Further statistical analysis was made in comparison of the variance ratios of blotter versus rolled towel by sample across labs. Variance ratios indicate that less variation between labs occurred when rolled towels were used than when blotters were used. The variance ratio is determined by dividing the variance found with the theoretical minimum variance, Table 5.

TABLE 1. SAMPLE 1 GERMINATION PERCENTAGES

LAB	BLOTTER	ROLLED TOWEL	LAB
1	98	97	7
		+1S------	
6	97	96	16
20	97	96	19
	+1S------		
17	96	96	25
4	95	95	1
10	95	95	3
3	94	95	6
8	94	95	8
9	94	95	17
25	94	94	2
23	93	94	23
2	92	94	26
	A--------		
13	92	93	4
14	91	93	13
		A--------	
19	91	93	14
12	90	93	20
21	90	92	9
26	90	92	10
16	89	92	12
	-1S------	-1S------	
18	85	89	21
	-2S------	-2S------	
	-3S------	-3S------	
7	77	83	18
AVERAGE	92%	93%	
RANGE	21%	14%	
S - STANDARD DEVIATION	4.7	3.0	

TABLE 2. SAMPLE 2 GERMINATION PERCENTAGES

LAB	BLOTTER	ROLLED TOWEL	LAB
20	95	88	2
	+2S------		
8	89	87	26
		+1S------	
9	89	85	3
	+1S------		
17	87	85	8
10	86	85	9
3	85	85	23
26	85	83	7
2	83	80	6
19	83	80	17
4	82	79	10
		A--------	
21	81	79	19
	A--------		
23	79	78	4
13	78	78	20*
16	77	78	25
6	75	76	14
1	74	75	1
25**	74	75	21
7	73	74	16
	-1S------	-1S------	
18	72	71	18
		-2S------	
12	71	65	13
14	69	63	12
AVERAGE	80%	79%	
RANGE	26%	25%	
S - STANDARD DEVIATION	7.0	6.7	

*Germination invalid due to replication differences.
**Germination based on 300 seeds due to replication differences.

TABLE 3. SAMPLE 3 GERMINATION PERCENTAGES

LAB	BLOTTER	ROLLED TOWEL	LAB
17	98	97	4
	+1S------		
1	97	97	17
4	97	97	23
		+1S------	
20	97	96	1
9	96	96	7
13	96	96	14**
6	95	95	6
8	95	95	8
10	95	95	13
2	94	95	19
3	94	95	26
21	A---94---	94	9
23	94	94	12
		A--------	
25	94	94	16
7	93	94	25
26	93	93	2
14	91	93	3
	-1S------		
16	90	93	20
		-1S------	
19	90	91	10
		-2S------	
18	88	87	21
	-2S------		
12	84	86	18
AVERAGE	94%	94%	
RANGE	14%	11%	
S - STANDARD DEVIATION	3.4	2.9	

**Germination based on 3 replications due to replication differences.

Table 4. REVISED ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Treatments				
Laboratory (L)	20	4,824.14	241.21	2.39**
Substrate (Su)	1	0.34	0.34	0.34
Error A (L X Su)	20	2,019.20	100.96	2.71
Sample (Sa)	2	21,803.93	10,901.97	293.30**
Sa X L	40	3,376.82	84.42	2.27**
Sa X Su	2	186.38	93.19	2.71**
Error B (Sa X L X Su)	40	1,488.20	37.21	3.22**
Error C (Replications + Reps. x All)	378	4,364.75 (8.12) (4,356.63)	11.55	

** Significant at the 1% level of probability.

Table 5. COMPARISON OF VARIANCE RATIOS

Substrate	Samp.	Germ	Variance	Theoretical Minimum Variance	Variance Ratio
Blotter	1	92.85	10.24	1.66	6.17
Rolled Towel	1	93.95	3.52	1.42	2.48
Blotter	2	80.33	48.43	3.95	12.26
Rolled Towel	2	78.52	44.36	4.22	10.51
Blotter	3	94.05	7.00	1.40	5.00
Rolled Towel	3	94.74	2.54	1.25	2.03

Submitted By: Richard C. Lawson
Idaho State Seed Laboratory

PROPOSAL #5

RULES PROPOSAL

Addition of 25°C to testing temperature in Table 4.

PRESENT RULE (If new rule, state "New Rule")

Kind of seed	Substrata	Temp. °C	1st count days	Final count days	Additional Directions
Impatiens walleriana Hooker f. Impatiens	P	20	7	18	Light, new crop seed sensitive to higher temperatures

PROPOSED RULE (Exactly as it would appear in "Rules")

Kind of seed	Substrata	Temp. °C	1st count days	Final count days	Additional Directions
Impatiens walleriana Hooker f. Impatiens	P	20, 25	7	18	Light, new crop seed sensitive to higher temperatures

SUPPORTING EVIDENCE (Research data, literature citations, published papers, or other appropriate information)

Seven laboratories participated in a referee involving ten lots of Impatiens seed tested at 20°C and 25°C. The data collected from five laboratories is attached. Two laboratories experienced equipment failure during the test period so their results are not included. The laboratories participaitng in this referee were:

Goldsmith Seeds, Gilroy, CA
McKenzie Seeds, Brandon, Manitoba, Canada
New York Seed Testing Laboratory, Geneva, NY
Pan American Seed, West Chicago, IL
Ransom Seed Laboratory, Carpinteria, CA
Seed Testing of America, Longmont, CO
Vaughan's Seed Co., Downers Grove, IL

SUBMITTED BY (Name, complete address, and phone number)

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DATE OF PROPOSAL

October 30, 1995

Impatiens Referee

Temperature:	°C	20° %	25° %	20° %	25° %	20° %	25° %	20° %	25° %	20° %	25° %	20° %	25 %
Sample:	A	98.75	94.50	97.00	83.75	96.00	96.00	94.25	93.75	96.25	96.00	96.5	92.8***
	B	93.25	94.50	*	99.25	88.00	81.00	92.75	94.00	89.75	92.00	90.9	93.8
	C	93.50	94.00	95.75	99.50	98.00	96.00	93.50	93.25	90.75	95.00	94.3	95.6
	D	95.00	94.50	99.00	98.00	100.00	99.00	94.75	96.25	97.75	97.00	97.3	97.0
	E	88.50	86.50	84.50	96.75	92.00	93.00	86.75	80.50	93.00	92.00	89.0	89.8
	F	97.50	97.00	98.75	97.50	94.00	97.00	97.00	96.75	96.50	97.00	96.8	97.1
	G	84.50	86.00	89.00	96.75	91.00	91.00	90.50	90.25	90.25	91.50	89.1	91.1
	H	97.00	98.25	94.75	96.00	96.00	96.00	86.75	95.00	96.50	99.00	94.2	96.9
	I	96.25	94.50	93.75	94.25	95.00	98.00	97.75	99.00	94.00	97.00	95.4	96.6
	J	98.75	98.75	99.25	100.00	97.00	99.00	98.25	96.50	99.00	99.50	98.5	98.8
Laboratory		1		3**		5		6		8		Average %	

* data not reported.

**final counts taken 3 days earlier than other laboratories.

***average excluding laboratory 3 is (95.1%)

PROPOSAL #6

Rules Proposal

Kind of seed: Lettuce, *Lactuca sativa*

Present Rule: Seedling Evaluation Handbook, page 18
Abnormal Seedling Description
Root
- none.
- primary root tip blunt, swollen and discolored.
- primary root with splits or lesions.

Proposed Rule: Seedling Evaluation Handbook, page 18
Abnormal Seedling Description
Root
- none.
- primary root weak.
- primary root tip blunt, swollen and discolored.
- primary root with splits or lesions.

Reasons for the proposed change:

This proposal is the correction of an error which was made in the preparation of the Seedling Evaluation Handbook. Appendix 1 of the Rules (Seedling Descriptions) was replaced by the Seedling Evaluation Handbook in 1992. One of the objectives of the Seedling Evaluation Committee was to remove vague wording from the seedling descriptions. The description in Appendix 1 for lettuce included "primary root missing, damaged or weak". The term "damaged" was discussed within the Committee and it was decided to delete it. In deleting it, the word "weak" was deleted as well, unintentionally. Review of the drafts of the Handbook failed to notice the deletion and so the final version was published with this description missing. Following publication of the Handbook, laboratories working with lettuce noticed that "weak" was missing from the description and requested that it be put back in.

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October 1, 1993

PROPOSAL #7

RULES PROPOSAL

Proposal to restrict the use of the "Chemical test to distinguish sweetclover " (Rule 3.4) to determining the presence of yellow sweetclover in samples of white sweetclover.

PRESENT RULE

3.4. Chemical test to distinguish sweetclover.- ~~In determining admixtures~~ of yellow sweetclover (Melilotus officinalis) ~~and~~ white sweetclover (M. albus) at least 400 seeds shall be subjected to the chemical test as follows:

a. Preparation of test solution.- Add 3 grams of cupric sulfate ($CuSO_4$) to 30 ml of household ammonia (NH_4OH, approx. 4.8%) in a stoppered bottle to form the tetraamminecopper sulfate ($[Cu(NH_3)_4]SO_4$) solution used for this test. After mixing, a light blue precipitate of cupric hydroxide ($Cu(OH)_2$) should form. If no precipitate forms, add additional $CuSO_4$ until a precipitate appears. Since the strength of household ammonia can vary, formation of a precipitate indicates that a complete reaction has taken place between $CuSO_4$ and NH_4OH; otherwise fumes from excess ammonium hydroxide may cause eye irritation.

b. Preparation of seeds.- To insure imbibition, scratch, prick, or otherwise scarify the seed coats of the sweetclover seeds being tested. Imbibe seeds in water for 2 to 5 hours in a glass container.

c. Chemical reaction.- When seeds have imbibed, remove excess water and add enough test solution to cover the seeds. Seed coats of ~~yellow sweetclover will begin to stain dark brown to black; seed coats of white sweetclover will be olive or yellow-green.~~ Make the separation within 20 minutes, since the seed coats of white sweetclover will eventually turn black also.

d. Calculation of results.- Count the number of seeds which stain dark brown or black and divide by the total number of seeds tested; multiply by the pure seed percentage for Melilotus spp.; the result is the percentage of yellow sweetclover in the sample. The percentage of white sweetclover is found by subtracting the percentage of yellow sweetclover from the percentage of Melilotus spp. pure seed.

Example:
Pure Melilotus spp. = 98.76%
Number of seeds tested = 400
Number of seeds staining dark brown of black = 32
% Yellow sweetclover = (32/400) x 98.76% = 7.90%
% White sweetclover = 98.76% - 7.90% = 90.86%

3.4. Chemical test to distinguish sweetclover.- To determine the presence of yellow sweetclover (Melilotus officinalis) in samples of white sweetclover (M. albus) at least 400 seeds shall be subjected to the chemical test as follows:

a. Preparation of test solution.- Add 3 grams of cupric sulfate ($CuSO_4$) to 30 ml of household ammonia (NH_4OH, approx. 4.8%) in a stoppered bottle to form the tetraamminecopper sulfate ($[Cu(NH_3)_4]SO_4$) solution used for this test. After mixing, a light blue precipitate of cupric hydroxide ($Cu(OH)_2$) should form. If no precipitate forms, add additional $CuSO_4$ until a precipitate appears. Since the strength of household ammonia can vary, formation of a precipitate indicates that a complete reaction has taken place between $CuSO_4$ and NH_4OH; otherwise fumes from excess ammonium hydroxide may cause eye irritation.

b. Preparation of seeds.- To insure imbibition, scratch, prick, or otherwise scarify the seed coats of the sweetclover seeds being tested. Imbibe seeds in water for 2 to 5 hours in a glass container.

c. Chemical reaction.- When seeds have imbibed, remove excess water and add enough test solution to cover the seeds. Seed coats of white sweetclover will stain olive or yellow-green; seed coats of most yellow sweetclover will stain dark brown to black. Make the separation within 20 minutes, since the seed coats of white sweetclover will eventually turn black also.

d. Calculation of results.- Count the number of seeds which stain dark brown or black and divide by the total number of seeds tested; multiply by the pure seed percentage for Melilotus spp.; the result is the percentage of yellow sweetclover in the sample. The percentage of white sweetclover is found by subtracting the percentage of yellow sweetclover from the percentage of Melilotus spp. pure seed. This may be an under-estimation of the percentage of yellow sweetclover in the sample, because a small percentage of yellow sweetclover may fail to stain dark brown or black. For this reason, this test is not appropriate for testing yellow sweetclover for the presence of white sweetclover.

Example:
Pure Melilotus spp. = 98.76%
Number of seeds tested = 400
Number of seeds staining dark brown of black = 32
% Yellow sweetclover = (32/400) x 98.76% = 7.90%
% White sweetclover = 98.76% - 7.90% = 90.86%

SUPPORTING EVIDENCE

Report of the AOSA Sweetclover Chemical Test Subcommittee

Submitted by Richard C. Payne
September 15, 1993

Plants of yellow sweetclover samples were grown in field plots during the summer of 1992. It was not possible to harvest seeds from these plants during 1992 because of animal predation. The animal predation problem was resolved during the fall of 1992, and the plants successfully over-wintered. During the spring and summer of 1993, the flower color of these plants was verified as yellow. Seeds were harvested on the following three dates: June 30, 1993, July 8, 1993, and July 16, 1993. A portion of the sample harvested on each of the three dates was tested with the sweetclover chemical test by the Nebraska State Seed Laboratory (NE), the Central Seed Laboratory, Agriculture Canada (CAN), and the U.S., Federal Seed Laboratory (FSL). The results are listed below.

Harvest Date	Lab	% of seeds staining dark brown or black	% of seeds staining olive or yellow-green	No. of seeds tested
06/30/93	NE	68.50	31.50	400
	CAN	76.79	23.21	418
	FSL	78.50	21.50	400
	avg.	74.60	25.40	
07/08/93	NE	84.25	15.75	400
	CAN	80.93	19.07	472
	FSL	86.00	14.00	400
	avg.	83.73	16.27	
07/16/93	NE	88.50	11.50	400
	CAN	96.36	3.64	550
	FSL	99.00	1.00	400
	avg.	93.62	5.38	

Observations

The results of this study show that the percentage of seeds staining dark brown or black, characteristic of yellow sweetclover, increased progressively after the first harvest date. This raises the possibility that the more mature yellow sweetclover seeds stained dark brown or black while the less mature seeds did not develop the dark color and appeared olive or yellow-green characteristic of white sweetclover.

Most seed pods from the first harvest on June 30, 1993, were tan while those from the later harvests were darker brown. Also, many seeds from the early harvest had greenish color in their seed coats. Very few seeds from the last harvest had greenish seed coats. This observation supports the contention that a number of seeds from the first harvest were less mature than seeds from the later harvests. If this is the case, immature yellow sweetclover seeds could be mistakenly identified as white sweetclover with the sweetclover chemical test.

We plan to retest these samples in several months to determine if there is a change in test results related to time in storage.

Suggestions

1. Apply a tolerance to the results of the sweetclover chemical test when testing yellow sweetclover samples. This tolerance would compensate for a certain percentage of yellow sweetclover seeds that may stain like white sweetclover seeds.

2. Use the sweetclover chemical test as a screening procedure when testing yellow sweetclover samples. If the results of the chemical test indicate that there is a lower percentage of yellow sweetclover in a sample than expected, an additional growout test to determine flower color could be made in a greenhouse or growth chamber.

3. Restrict the use of the sweetclover chemical test to testing white sweetclover samples for darker staining yellow sweetclover seeds. This is the same way in which the "mottled seed examination" was used in the past.

A retest (Test 2) of the yellow sweetclover samples was completed November 19, 1993, at the Federal Seed Laboratory. The results are listed below:

Harvest Date	Test	% of seeds staining dark brown or black	% of seeds staining olive or yellow-green	No. of seeds tested
06/30/93	1	78.50	21.50	400
	2	81.50	18.50	400
07/08/93	1	84.25	15.75	400
	2	86.50	13.50	400
07/16/93	1	99.00	1.00	400
	2	94.25	5.75	400

References for the sweetclover chemical test:

Maxon, S.R. and S.J. Hurst. 1983. A comparison of methods to distinguish seeds of yellow sweetclover (Melilotus officinalis (L.) Lam.) and white sweetclover (M. alba Medik.) AOSA Newsletter 57 (1):46-53.

Maxon, S.R. 1985. Rule Proposal 10. Chemical test to distinguish seetclover. AOSA Newsletter 59(1):28-29.

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