Rule Change Proposal 12 - AMENDED

Purpose of the Rule: The purpose of this rule is to change germination procedures for butterfly milkweed, *Asclepias tuberosa* L.

Present Rule:

Kind of Seed	Substrate[a]	Temperature (C°)	First Count (days)	Final Count (days)	Specific requirements	Fresh and Dormant
Asclepias tuberosa butter-fly weed, butterfly milkweed	P	10-30	6[b]	14c	Light. Prechill 21 days at 3-5°C prior to testing	

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

f Dormant seed: Expected dormant, either short term (due to recent harvest) or long term dormancy.

Proposed Rule:

Kind of Seed	Substrate[a]	Temperature (C°)	First Count (days)	Final Count (days)	Specific requirements	Fresh and Dormant
Asclepias tuberosa butter-fly weed, butterfly milkweed	P	20-30	7[b]	14[c]	~~KNO_3~~ Light. Prechill 14 days at 3-5°C. Ungerminated seeds: see sec. 6.2f and 6.9m	

a For coated seed, pleated paper (PP) may be used instead of the listed substrata. See sec. 6.8 l (1).
b Make preliminary counts only when necessary or advisable for efficiency. Many flower seedlings can be judged more accurately and critically if seedlings are left for final count, especially in test durations of 7-14 days.
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Harmonization and Impact Statement: *Asclepias tuberosa* L. is not recognized in the Federal Seed Act, or the Canadian Methods or Procedures. ISTA tests the species at 20-30C for 28 days. This would bring the temperature option in harmonization with ISTA.

Supporting Evidence:

Materials and methods:

Three lots of *Asclepias tuberosa* were obtained from companies with various dormancy levels and geographical locations. First the lots were tested in house to determine if the current germination

temperature is still the best procedure. Due to the high price and high demand for the seed large quantities were not available. Two lots were randomly selected and were planted in two replicates of 50 seeds. Each lot was planted using KNO_3 with a one week, two week, and four week pre-chill. Each lot was then moved into 15-25C, 10-30C, 20-30C, and 20-35C for a total of 12 different germination/dormancy breaking combinations. Counts were done at 7, 14, 21, and 28 days. At the end of the test the firm ungerminated seeds were checked for dormancy using the TZ method.

This in-house experiment was used to narrow down the procedures used to compare to the current method for a lab referee. The in-house method that appeared to achieve the best results with a two week pre-chill at 20-30C.

Eight labs were then sent three lots of seed to test according to the current method and the proposed method of 20-30C. They were asked to do counts at 7 and 14 days. They were also asked to determine dormancy at the end of the testing period.

Research through the USDA plant database and current Asclepias rules recommended 3-5C as the appropriate pre-chill method. Some of the research even recommended using down to 1C. Research published in the AOSA newsletter volume 47 issue 1 showed that they had the best success with a 3-5C prechill.

Results:

In-house testing showed that no matter the length of pre-chill or the germination temperature there was not significant growth between 21 to 28 days. The final count days are recommended to be 21 days. At the 10-30C germination temperature (Table 2), the growth was slow to start with a one week pre-chill. The best growth at the first count was with a four week pre-chill. At the 15-25C germination temperature (Table 1), the best germination was obtained with a one week pre-chill for lot a and a two week pre-chill for lot b. At the 20-30C germination temperature (Table 3), all lots had significant growth at the first count no matter the length of pre-chill. Maximum growth was obtained in seven days on both lots. At the 20-35C germination temperature (Table 4), all three pre-chill lengths produced very similar results and maximum germination was achieved in seven days on both lots.

Table 5 shows the average germination, abnormals, dead, dormant, and total viable for each temperature/pre-chill regime. On lot A the total viability ranged from a 78% to a 92% across all methods (Table 6). On lot B the total viability ranged from a 77% to a 91% (Table 7). Of the four germination temperatures tested in-house the 20-30C produced the highest germination results the quickest. Neither of the two lots had significant dormancy. The lots obtained were labelled to contain dormant seeds.

Eight laboratories returned results. Lab 3 stated that their samples were potentially higher in dormancy due to some of the replicates lacking in moisture.

The proposed method of 20-30C with a two week pre-chill presented with significantly higher first counts than the current method of 10-30C with a three week pre-chill. The proposed and current method were not significantly different from one another at the final count (Table 8).

Tables 9-11 show the results for the eight laboratories using the current testing method for lots a, b, and c. Lab 2 appeared to be tougher on the evaluation as they were typically higher in abnormals than the other seven laboratories. If laboratory two's data is removed the spread between the minimum and maximum really narrows done. Laboratories are evaluating the samples very similar and the results appear to be repeatable amongst labs.

Tables 12-14 show the results for the eight laboratories using the proposed testing methods for lots a, b, and c. Again lab 2 typically had higher abnormals than the other seven laboratories.

For lot a current and proposed methods performed equally well. Four laboratories received higher results at the current method of 10-30C and four laboratories received higher results with the proposed method of 20-30C. For lot b the proposed method received higher results for five of the laboratories than the current method. For lot c, five laboratories received higher results for the

current method than the two for the proposed method. When the current method was higher than the proposed method the average percentage increase was 4%. When the proposed method was higher than the current method the average percentage increase was 3%.

The variability among labs is more than the variability among methods.

Statistically based off the data received, there is no significant difference between the current method and the proposed method of using 20-30C. It is being recommended to amend the current method of 10-30C to the proposed method of 20-30C. Statistically the proposed method presented with higher first counts and 20-30C is a more universal temperature used for all laboratories than 10-30C. This will also decrease the germination testing period by seven days without compromising results. This will also put us closer into harmonization with ISTA.

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Appendix

Table 1. Germination counts for the 2 lots tested in-house at 15-25C with 3-5C prechill

15-25C	1st count (7 day)	2nd count (14 day)	3rd count (21 day)	Final count (28 day)	Total Germ	abs	dead	dormant
1 week prechill 15-25								
a	80	8	0	0	88	2	10	0
b	75	2	0	0	77	2	21	0
2 week prechill 15-25								
a	78	3	0	0	81	0	19	0
b	80	2	0	0	82	0	18	0
4 week prechill 15-25								
a	83	0	0	0	83	2	14	1
b	81	0	0	0	81	1	18	0

Table 2. Germination counts for the two lots tested in-house at 10-30C with 3-5C prechill

10-30C	1st count (7 day)	2nd count (14 day)	3rd count (21 day)	Final count (28 day)	Total Germ	abs	dead	dormant
1 week prechill 10-30								
a	0	79	3	0	82	4	14	0
b	0	75	5	0	80	3	17	0
2 week prechill 10-30								
a	75	6	0	0	81	3	16	0
b	78	3	0	0	81	2	17	0
4 week prechill 10-30								
a	77	0	0	0	77	5	17	1
b	80	0	0	0	80	3	17	0

Table 3. Germination counts for the two lots tested in-house at 20-30C with 3-5C prechill

20-30C	1st count (7 day)	2nd count (14 day)	3rd count (21 day)	Final count (28 day)	Total Germ	abs	dead	dormant
1 week prechill 20-30								
a	90	0	0	0	90	2	8	0
b	89	0	0	0	89	1	10	0
2 week prechill 20-30								
a	89	0	0	0	89	0	8	3
b	91	0	0	0	91	0	9	0
4 week prechill 20-30								
a	85	0	0	0	85	2	13	0
b	84	0	0	0	84	3	13	0

Table 4. Germination counts for the two lots tested in-house at 20-35C with 3-5C prechill

	1st count (7 day)	2nd count (14 day)	3rd count (21 day)	Final count (28 day)	Total Germ	abs	dead	dormant
1 week prechill 20-35	88	0	0	0	88	1	11	0
a	85	0	0	0	85	3	12	0
b								
2 week prechill 20-35								
a	84	0	0	0	84	1	13	1
b	88	0	0	0	88	1	11	0
4 week prechill 20-35								
a	86	0	0	0	86	0	14	0
b	84	0	0	0	94	0	16	0

Table 5. Average germination, abnormals, dead, dormant and total viable for each in-house regime.

	Germ	Abs	Dead	Dormant	Viable
A 15-25 1 week prechill	88	2	10	0	88
B 15-25 1 week prechill	77	2	21	0	77
A 15-25 2 week prechill	81	0	19	0	81
B 15-25 2 week prechill	82	0	18	0	82
A 15-25 4 week prechill	83	2	14	1	84
B 15-25 4 week prechill	81	1	18	0	81
A 10-30 1 week prechill	82	4	14	0	82
B 10-30 1 week prechill	80	3	17	0	80
A 10-30 2 week prechill	81	3	16	0	81
B 10-30 2 week prechill	81	2	17	0	81
A 10-30 4 week prechill	77	5	17	1	78
B 10-30 4 week prechill	80	3	17	0	80
A 20-30 1 week prechill	90	2	8	0	90
B 20-30 1 week prechill	89	1	10	0	89
A 20-30 2 week prechill	89	0	8	3	92
B 20-30 2 week prechill	91	0	9	0	91
A 20-30 4 week prechill	85	2	13	0	85
B 20-30 4 week prechill	84	3	13	0	84
A 20-35 1 week prechill	88	1	11	0	88
B 20-35 1 week prechill	85	3	12	0	85
A 20-35 2 week prechill	84	1	13	1	85
B 20-35 2 week prechill	88	1	11	0	88
A 20-35 4 week prechill	86	0	14	0	86
B 20-35 4 week prechill	84	0	16	0	84

Table 6. Lot A sorted by viability across all in-house regimes.

	Germ	Abs	Dead	Dormant	Viable
A 20-30 2 week prechill	89	0	8	3	92
A 20-30 1 week prechill	90	2	8	0	90
A 15-25 1 week prechill	88	2	10	0	88
A 20-35 1 week prechill	88	1	11	0	88
A 20-35 4 week prechill	86	0	14	0	86
A 20-30 4 week prechill	85	2	13	0	85
A 20-35 2 week prechill	84	1	13	1	85
A 15-25 4 week prechill	83	2	14	1	84
A 10-30 1 week prechill	82	4	14	0	82
A 10-30 2 week prechill	81	3	16	0	81
A 15-25 2 week prechill	81	0	19	0	81
A 10-30 4 week prechill	77	5	17	1	78

Table 7. Lot B sorted by viability across all in-house regimes.

	Germ	Abs	Dead	Dormant	Viable
B 20-30 2 week prechill	91	0	9	0	91
B 20-30 1 week prechill	89	1	10	0	89
B 20-35 2 week prechill	88	1	11	0	88
B 20-35 1 week prechill	85	3	12	0	85
B 20-30 4 week prechill	84	3	13	0	84
B 20-35 4 week prechill	84	0	16	0	84
B 15-25 2 week prechill	82	0	18	0	82
B 10-30 2 week prechill	81	2	17	0	81
B 15-25 4 week prechill	81	1	18	0	81
B 10-30 1 week prechill	80	3	17	0	80
B 10-30 4 week prechill	80	3	17	0	80
B 15-25 1 week prechill	77	2	21	0	77

Table 8. Statistical analysis of the proposed method and the current method.

Method	1ST COUNT	FINAL COUNT	ABS	DEAD	DORMANT	Total Viable	1ST COUNT	FINAL COUNT	Total Viable
Proposed (20-30C)	64.79	82.77	4.333	12.5	0.3854	83.16	B	NS	NS
Current (10-30C)	53.66	83.13	2.542	13.7	0.5521	83.68	A	NS	NS
							P=0.0000	P=0.8749	P=0.9456
							LSD (0.05) = 0.8852	LSD (0.05) = 0.9140	LSD (0.05) = 0.9037
								NS= not significant	

Table 9. Germination results for Lot a with the current testing method

Lot a	normal	abs	dead	dormant	total viable
LAB 1	91	1	8	0	91
LAB 2	76	7	17	0	76
LAB 3	85	0	12	3	88
LAB 4	87	0	13	0	87
LAB 5	85	1	13	1	86
LAB 6	85	3	12	0	85
LAB 7	85	3	11	1	86
LAB 8	89	4	7	0	89
Max	91	7	17	3	91
Min	76	0	7	0	76
Average	85.375	2.375	11.625	0.625	86
STDV	4.4057592	2.3867192	3.1139089	1.0606602	4.472136

Table 10. Germination results for lot b with the current testing method

Lot b	normal	abs	dead	dormant	total viable
LAB 1	89	2	7	2	91
LAB 2	78	8	14	0	78
LAB 3	85	0	12	3	88
LAB 4	87	0	13	0	87
LAB 5	85	2	12	1	86
LAB 6	83	2	15	0	83
LAB 7	83	3	14	0	83
LAB 8	84	6	10	0	84
Max	89	8	15	3	91
Min	78	0	7	0	78
Average	84.25	2.875	12.125	0.75	85
STDV	3.2403703	2.7998724	2.5877458	1.1649647	3.927922

Table 11. Germination results for lot c with the current testing method

Lot c	normal	abs	dead	dormant	total viable
LAB 1	87	1	10	2	89
LAB 2	70	5	25	0	70
LAB 3	82	0	17	1	83
LAB 4	80	1	18	1	81
LAB 5	83	1	16	0	83
LAB 6	80	3	17	0	80
LAB 7	80	7	13	0	80
LAB 8	84	4	12	0	84
Max	87	7	25	2	89
Min	70	0	10	0	70
Average	80.75	2.75	16	0.5	81.25
STDV	4.9785253	2.4348658	4.5981363	0.7559289	5.3917927

Table 12. Germination results for lot a with the proposed testing method

Lot a	normal	abs	dead	dormant	total viable
LAB 1	91	1	7	1	92
LAB 2	69	21	10	0	69
LAB 3	92	0	8	0	92
LAB 4	82	1	17	0	82
LAB 5	87	3	10	0	87
LAB 6	89	2	9	0	89
LAB 7	82	7	11	0	82
LAB 8	83	6	11	0	83
Max	92	21	17	1	92
Min	69	0	7	0	69
Average	84.375	5.125	10.375	0.125	84.5
STDV	7.3666721	6.8751623	3.0207615	0.3535534	7.5023806

Table 13. Germination results for lot b with the proposed testing method

Lot b	normal	abs	dead	dormant	total viable
LAB 1	90	1	6	3	93
LAB 2	75	12	13	0	75
LAB 3	90	0	10	0	90
LAB 4	88	1	11	0	88
LAB 5	85	3	12	0	85
LAB 6	91	2	7	0	91
LAB 7	81	6	13	0	81
LAB 8	85	4	11	0	85
Max	91	12	13	3	93
Min	75	0	6	0	75
Average	85.625	3.625	10.375	0.375	86
STDV	5.449443	3.8890873	2.6152028	1.0606602	5.8797473

Table 14. Germination results for lot c with the proposed testing method

Lot c	normal	abs	dead	dormant	total viable
LAB 1	87	1	10	2	89
LAB 2	66	18	16	0	66
LAB 3	86	0	13	1	87
LAB 4	71	1	28	0	71
LAB 5	80	3	17	0	80
LAB 6	84	1	15	0	84
LAB 7	74	8	18	0	74
LAB 8	79	6	15	0	79
Max	87	18	28	2	89
Min	66	0	10	0	66
Average	78.375	4.75	16.5	0.375	78.75
STDV	7.5011904	6.041523	5.2644359	0.7440238	7.9955345

Table 15. Analysis of variance for the total viability of seeds and lab-to lab variation on the germination of three milkweed samples tested at 8 laboratories using the same germination parameters

SOURCE	DF	S.S	M.S	F VALUE	P VALUE
LOTS	2	463.14	381.57	38.03	0.0000
METHOD	1	0.05	0.05	0.00	0.9456
LOT X METHOD	2	76.59	38.30	3.82	0.0243
LAB	7	3177.40	453.91	45.24	0.0000
LOT X LAB	14	114.11	8.15	0.81	0.6548
METHOD X LAB	7	410.49	58.64	5.84	0.0000
LOT X METHOD X LAB	14	111.49	28.19	7.96	0.6748