

## Rule Change Proposal 11

**Purpose of the Rule:** The purpose of this rule is to add germination procedures for common milkweed, *Asclepias syriaca* L.

**Present Rule:** New Rule

**Proposed Rule:**

Kind of Seed	Substrate <sup>a</sup>	Temperature (C°)	First Count (days)	Final Count (days)	Specific requirements	Fresh and Dormant
<i>Asclepias syriaca</i> L., common milkweed	P	15-25; 20-30	7 <sup>b</sup>	21 <sup>c</sup>	KNO <sub>3</sub> Prechill 14 days at 3-5C. Ungerminated seeds: see sec. 6.2f and 6.9m	

<sup>a</sup> For coated seed, pleated paper (PP) may be used instead of the listed substrata. See sec. 6.8 I (1).

<sup>b</sup> 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.

<sup>c</sup> 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).

<sup>d</sup> Avoid injury to the radicle or other embryo structures.

<sup>e</sup> Rhizomatous derivatives of a johnsongrass x sorghum cross or a johnsongrass x sudangrass cross.

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

**Harmonization and Impact Statement:** *Asclepias syriaca* L. is not recognized in the Federal Seed Act, the Canadian Methods or Procedures, or ISTA Seed Testing Rules.

### Supporting Evidence:

Materials and methods:

Four lots of *Asclepias syriaca* were obtained from companies with various dormancy levels and geographical locations. First the lots were tested in house to try to narrow down the germination procedures. 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<sub>3</sub> 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.

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.

This in-house experiment was used to narrow down the procedures used to send out to laboratories for a lab referee.

Ten labs were then sent four lots of seed to test according to the best two in-house methods. They were asked to do counts at 7, 14, and 21 days. They were also asked to determine dormancy at the end of the testing period.

Results:

In-house testing showed that the only temperature regime that had significant growth between 21 and 28 days was at 10-30C. The final count days are recommended to be 21 days. At the 15-25C germination temperature (Table 1), the fastest rate of growth was with the two week pre-chill. The slowest rate of growth was with the one week pre-chill. At the 10-30C germination

temperature (Table 2), no matter the length of pre-chill, the growth was slow to start. The best growth at the first count was with a four week pre-chill. At the 20-30C germination temperature (Table 3), all lots had significant growth at the first count no matter the length of pre-chill. The best first count germination was with a two week pre-chill. At the 20-35C germination temperature (Table 4), there was significant growth with the four week pre-chill.

Table 5 shows the average germination, abnormal, dead, dormant, and total viable for each temperature/pre-chill regime. On lot A the total viability ranged from an 83% to a 96% across all methods (Table 6). On lot B the total viability ranged from a 62% to a 77% (Table 7). The 10-30C germination temperature option was removed from the lab referee because it had the highest amount of dormancy remaining across all pre-chill lengths and temperature options. The one week pre-chill showed the highest dormancy across the temperature regimes so it appears that one week is not long enough. The 20-35C temperature regime also appears to still show a significantly higher amount of dormancy than 15-25C and 20-30C. The 20-30C temperature regime still shows some dormancy but significantly less than 10-30C and 20-35C. The 15-25C temperature combination showed the least amount of dormancy remaining at the end of the testing period. 15-25C appeared to provide the best germination temperature with the least amount of dormancy followed by 20-30C. These two temperature regimes along with a two week pre-chill were recommended to be sent out for comparison amongst labs to determine if 15-25C is significantly better.

Nine of the ten laboratories returned results. Lab 2 informed that some results maybe higher in dormancy due to lack of moisture during the testing period.

The proposed method of 15-25C with a two week pre-chill presented with higher final count and total viable than the proposed method of 20-30C with a two week pre-chill. The 15-25C method is statistically different than the 20-30C method. The two results are within tolerance of one another (Table 8).

Tables 9-12 show the results among the nine laboratories using the proposed method of 15-25C with a two week pre-chill for lots a, b, c, and d. Labs one, seven and nine appeared to be tougher on evaluation because they have a higher percentage of abnormal than the other laboratories most of the time.

Tables 13-16 show the results among the nine laboratories using the proposed method of 20-30C with a two week pre-chill for lots a, b, c, and d. Labs one, seven, and nine again appeared to be harder on evaluation because they have a higher percentage of abnormal than the other laboratories most of the time.

For lot a 15-25C method achieved higher viability for five laboratories than two for 20-30C. Two laboratories had the same viability for both methods. For lot b the 15-25C method achieved higher viability for five laboratories versus four for 20-30C. For lot c the 15-25C method achieved higher results than two labs for 20-30C. Three labs had equal viability between the two methods as well. For lot d, five laboratories achieved higher viability than three labs at the 20-30C method. One laboratory had equal viability between both methods. When the 15-25C method is higher than the 20-30C method, on average it is by 5%. When the 20-30C method is higher than the 15-25C method, it is by 3% on average.

Statistically the total viable is significantly different in 15-25 than the 20-30C when it comes to the total viable and final count. The 20-30C early counts are significantly different than the 15-25C. The 20-30C produces a quicker germination than the 15-25C. The 15-25C produces a slightly higher total viable percentage. The total viable for the 15-25C method was 79.55% and the total viable for the 20-30C method is a 78.16%. These two results are well within tolerance of one another and appears that both would provide accurate and useable results. The variability among labs is more than the variability among methods.

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## Appendix

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

15-25C	1st count (7 day)	2nd count (14 day)	3rd count (21 day)	Final count (28 days)	Total Germ	abs	dead	dormant
<b>1 week prechill 15-25</b>								
<b>a</b>	35	49	0	0	84	1	15	1
<b>b</b>	20	48	1	0	69	2	29	0
<b>2 week prechill 15-25</b>								
<b>a</b>	72	17	4	0	93	0	7	0
<b>b</b>	52	22	0	0	74	2	23	1
<b>4 week prechill 15-25</b>								
<b>a</b>	72	11	0	0	83	8	9	0
<b>b</b>	45	16	2	0	63	3	34	0

Table 2. Germination counts for the 2 in house tested lots at 10-30C with a 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
<b>1 week prechill 10-30</b>								
<b>a</b>	0	22	16	10	48	4	9	39
<b>b</b>	0	10	20	14	44	3	25	28
<b>2 week prechill 10-30</b>								
<b>a</b>	10	39	13	0	62	1	12	25
<b>b</b>	5	25	10	0	40	2	18	20
<b>4 week prechill 10-30</b>								
<b>a</b>	26	31	8	0	65	0	15	20
<b>b</b>	16	20	5	1	42	4	18	24

Table 3. Germination counts for the 2 in house tested lots at 20-30C with a 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
<b>1 week prechill 20-30</b>								
<b>a</b>	70	8	2	1	81	0	7	12
<b>b</b>	48	10	2	1	61	1	29	16
<b>2 week prechill 20-30</b>								
<b>a</b>	88	3	0	0	91	0	4	5
<b>b</b>	55	14	3	0	72	3	23	2
<b>4 week prechill 20-30</b>								
<b>a</b>	84	0	0	0	84	1	12	3
<b>b</b>	50	12	0	0	62	2	29	7

Table 4. Germination counts for the 2 in house tested lots at 20-35C with a 3-5C prechill

20-35C	1st count (7 day)	2nd count (14 day)	3rd count (21 day)	Final count (28 day)	Total Germ	abs	dead	dormant
<b>1 week prechill 20-35</b>								
<b>a</b>	55	18	2	0	75	0	12	13
<b>b</b>	38	15	1	0	54	2	32	12
<b>2 week prechill 20-35</b>								
<b>a</b>	64	6	1	0	71	1	12	16
<b>b</b>	46	10	4	0	60	1	31	8
<b>4 week prechill 20-35</b>								
<b>a</b>	86	0	0	0	86	0	11	3
<b>b</b>	49	8	0	0	57	1	32	10

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

	Germ	Abs	Dead	Dormant	Viable
<b>A 15-25 1 week prechill</b>	84	1	15	1	85
<b>B 15-25 1 week prechill</b>	69	2	29	0	69
<b>A 15-25 2 week prechill</b>	93	0	7	0	93
<b>B 15-25 2 week prechill</b>	74	2	23	1	65
<b>A 15-25 4 week prechill</b>	83	8	9	0	83
<b>B 15-25 4 week prechill</b>	63	3	34	0	63
<b>A 10-30 1 week prechill</b>	48	4	9	39	87
<b>B 10-30 1 week prechill</b>	34	3	25	28	62
<b>A 10-30 2 week prechill</b>	62	1	12	25	87
<b>B 10-30 2 week prechill</b>	40	1	12	25	65
<b>A 10-30 4 week prechill</b>	65	0	15	20	85
<b>B 10-30 4 week prechill</b>	42	4	18	24	66
<b>A 20-30 1 week prechill</b>	81	0	7	12	93
<b>B 20-30 1 week prechill</b>	61	1	29	16	77
<b>A 20-30 2 week prechill</b>	91	0	4	5	96
<b>B 20-30 2 week prechill</b>	72	3	23	2	74
<b>A 20-30 4 week prechill</b>	84	1	12	3	87
<b>B 20-30 4 week prechill</b>	62	2	29	7	69
<b>A 20-35 1 week prechill</b>	75	0	12	13	88
<b>B 20-35 1 week prechill</b>	54	2	32	12	66
<b>A 20-35 2 week prechill</b>	71	1	12	16	87
<b>B 20-35 2 week prechill</b>	60	1	31	8	68
<b>A 20-35 4 week prechill</b>	86	0	11	3	89
<b>B 20-35 4 week prechill</b>	57	1	32	10	67

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

	Germ	Abs	Dead	Dormant	Viable
A 20-30 2 week prechill	91	0	4	5	96
A 15-25 2 week prechill	93	0	7	0	93
A 20-30 1 week prechill	81	0	7	12	93
A 20-35 4 week prechill	86	0	11	3	89
A 20-35 1 week prechill	75	0	12	13	88
A 10-30 1 week prechill	48	4	9	39	87
A 10-30 2 week prechill	62	1	12	25	87
A 20-30 4 week prechill	84	1	12	3	87
A 20-35 2 week prechill	71	1	12	16	87
A 10-30 4 week prechill	65	0	15	20	85
A 15-25 1 week prechill	84	1	15	1	85
A 15-25 4 week prechill	83	8	9	0	83

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

	Germ	Abs	Dead	Dormant	Viable
B 20-30 1 week prechill	61	1	29	16	77
B 15-25 2 week prechill	74	2	33	1	75
B 20-30 2 week prechill	72	3	23	2	74
B 15-25 1 week prechill	69	2	29	0	69
B 20-30 4 week prechill	62	2	29	7	69
B 20-35 2 week prechill	60	1	31	8	68
B 20-35 4 week prechill	57	1	32	10	67
B 10-30 4 week prechill	42	4	18	24	66
B 20-35 1 week prechill	54	2	32	12	66
B 10-30 2 week prechill	40	1	12	25	65
B 15-25 4 week prechill	63	3	34	0	63
B 10-30 1 week prechill	34	3	25	28	62

Table 8. Statistical analysis of the proposed method #1 15-25C and proposed method #2 20-30C.

	Method 1 (15-25C)	Method 2 (20-30C)		
1st count	29.82a	61.88b	P=0.0000	LSD (0.05) = 0.7220
2nd count	71.80a	74.80b	P=0.0000	LSD (0.05) = 0.8263
Final count	78.23a	75.73b	P=0.0000	LSD (0.05) = 0.7343
Abs	4.56a	4.66b		
Deads	15.92a	17.16b		
Dormant	1.33 a	2.43b		
Total Viable	79.55a	78.16b	P=0.0091	LSD (0.05) = 0.7067

Table 9. Germination results for lot a with proposed method of 15-25C with 2 week pre-chill 3-5C

Lot a	normal	abs	dead	dormant	total viable
LAB 1	62	3	35	0	62
LAB 2	62	0	33	5	67
LAB 3	62	2	29	7	69
LAB 4	68	1	31	0	68
LAB 6	67	3	30	0	67
LAB 7	50	16	33	1	51
LAB 8	61	1	36	2	63
lab 9	56	8	36	0	56
lab 10	67	4	27	2	69
Max	68	16	36	7	69
Min	50	0	27	0	51
Average	61.6666667	4.22222222	32.2222222	1.88888889	63.5555556
STDV	5.7662813	4.99444135	3.19287401	2.52212433	6.32675097

Table 10. Germination results for lot b with proposed method of 15-25C with 2 week pre-chill 3-5C

Lot b	normal	abs	dead	dormant	total viable
LAB 1	78	9	13	0	78
LAB 2	86	0	12	2	88
LAB 3	88	1	10	1	89
LAB 4	86	1	13	0	86
LAB 6	79	2	19	0	79
LAB 7	70	16	13	1	71
LAB 8	83	1	15	1	84
lab 9	77	10	13	0	77
lab 10	85	6	9	0	85
Max	88	16	19	2	89
Min	70	0	9	0	71
Average	81.3333333	5.11111111	13	0.55555556	81.8888889
STDV	5.78791845	5.53273089	2.87228132	0.72648316	5.9675046

Table 11. Germination results for lot c with proposed method of 15-25C with 2 week pre-chill 3-5C

Lot c	normal	abs	dead	dormant	total viable
LAB 1	83	4	13	0	83
LAB 2	84	0	10	6	90
LAB 3	93	0	5	2	95
LAB 4	91	2	7	0	91
LAB 6	89	2	9	0	89
LAB 7	83	6	9	2	85
LAB 8	91	1	8	0	91
lab 9	84	8	8	0	84
lab 10	84	5	9	2	86
Max	93	8	13	6	95
Min	83	0	5	0	83
Average	86.8888889	3.11111111	8.66666667	1.33333333	88.2222222
STDV	4.04488703	2.80376731	2.17944947	2	3.96162144

Table 12. Germination results for lot d with proposed method of 15-25C with 2 week pre-chill 3-5C

Lot d	normal	abs	dead	dormant	total viable
LAB 1	84	6	10	0	84
LAB 2	89	0	6	5	94
LAB 3	82	4	10	2	84
LAB 4	89	3	8	0	89
LAB 6	84	5	11	0	84
LAB 7	80	10	9	1	81
LAB 8	85	3	11	1	86
lab 9	82	12	6	0	82
lab 10	81	9	10	0	81
Max	89	12	11	5	94
Min	80	0	6	0	81
Average	84	5.77777778	9	1	85
STDV	3.24037035	3.8658045	1.93649167	1.6583124	4.21307489

Table 13. Germination results for lot a with proposed method of 20-30C with 2 week pre-chill 3-5C

Lot a	normal	abs	dead	dormant	total viable
LAB 1	51	8	41	0	51
LAB 2	55	0	33	12	67
LAB 3	64	2	30	4	68
LAB 4	61	2	37	0	61
LAB 6	70	5	25	0	70
LAB 7	56	9	34	1	57
LAB 8	59	1	37	3	62
lab 9	52	5	39	4	56
lab 10	62	3	30	5	67
Max	70	9	41	12	70
Min	51	0	25	0	51
Average	58.8888889	3.88888889	34	3.22222222	62.1111111
STDV	6.09188896	3.10017921	5.07444578	3.83333333	6.45066749

Table 14. Germination results for lot b with proposed method of 20-30C with 2 week pre-chill 3-5C

Lot b	normal	abs	dead	dormant	total viable
LAB 1	74	14	12	0	74
LAB 2	86	0	8	6	92
LAB 3	87	2	11	0	87
LAB 4	83	3	14	0	83
LAB 6	83	2	15	0	83
LAB 7	60	27	13	0	60
LAB 8	74	3	21	2	76
lab 9	79	3	18	0	79
lab 10	85	4	10	1	86
Max	87	27	21	6	92
Min	60	0	8	0	60
Average	79	6.44444444	13.5555556	1	80
STDV	8.60232527	8.67627672	4.03457281	2	9.35414347

Table 15. Germination results for lot c with proposed method of 20-30C with 2 week pre-chill 3-5C

Lot c	normal	abs	dead	dormant	total viable
LAB 1	87	6	7	0	87
LAB 2	79	0	8	13	92
LAB 3	86	2	7	5	91
LAB 4	88	2	10	0	88
LAB 6	82	6	12	0	82
LAB 7	85	5	10	0	85
LAB 8	85	0	10	5	90
lab 9	78	4	12	6	84
lab 10	80	2	12	6	86
Max	88	6	12	13	92
Min	78	0	7	0	82
Average	83.3333333	3	9.77777778	3.88888889	87.2222222
STDV	3.67423461	2.34520788	2.04803429	4.40012626	3.34580998

Table 16. Germination results for lot d with proposed method of 20-30C with 2 week pre-chill 3-5C

Lot d	normal	abs	dead	dormant	total viable
LAB 1	75	15	10	0	75
LAB 2	86	0	9	5	91
LAB 3	89	3	8	0	89
LAB 4	87	4	9	0	87
LAB 6	80	5	15	0	80
LAB 7	80	8	12	0	80
LAB 8	86	3	10	1	87
lab 9	82	5	13	0	82
lab 10	84	7	7	2	86
Max	89	15	15	5	91
Min	75	0	7	0	75
Average	83.2222222	5.55555556	10.3333333	0.88888889	84.1111111
STDV	4.38114648	4.24591307	2.54950976	1.69148193	5.15859585

Table 17. Analysis of variance for the total viability of seeds and lab-to lab variation on the germination of four milkweed samples tested at 7 laboratories using the same germination parameters

SOURCE	DF	S.S	M.S	F VALUE	P VALUE
LOTS	3	12220.00	4073.20	498.85	0.0000
METHOD	1	57.11	57.11	6.95	0.0091
LOT X METHOD	3	10.75	3.58	0.44	0.7273
LAB	7	2886.40	412.34	50.20	0.0000
LOT X LAB	21	705.93	33.62	4.09	0.0000
METHOD X LAB	7	136.12	19.45	2.37	0.0243
LOT X METHOD X LAB	21	365.84	17.42	2.12	0.0043