

Rule Change Proposal 10

Purpose of the Rule: The purpose of this rule is to add germination procedures for showy milkweed, *Asclepias speciosa* Torr.

Present Rule: New Rule

Proposed Rule:

Kind of Seed	Substrate ^a	Temperature (C°)	First Count (days)	Final Count (days)	Specific requirements	Fresh and Dormant
<i>Asclepias speciosa</i> Torr., Showy milkweed	P	20-30	7 ^b	21 ^c	KNO ₃ Prechill 14 days at 3-5C. 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 I (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.

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

Supporting Evidence:

Materials and methods:

Seven lots of *Asclepias speciosa* 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 KNO₃ 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. It appeared based off the in-house testing that a two week pre-chill at 20-35C or 20-30C provided the best germination results with the least amount of dormancy. Seven labs were then sent the seven lots of seed to test according to these two 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 no matter the length of pre-chill or the germination temperature there was no significant growth between 21 and 28 days. The final count days are recommended to be 21 days. 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

15-25C germination temperature (Table 1), the fastest rate of growth was with the four week pre-chill. The slowest rate of growth was with the two 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. All pre-chill lengths showed significant root growth two days after moving into the germination chamber. At the 20-35C germination temperature (Table 4), there was significant growth with the 2 week and 4 week pre-chill for the one lot and at the 4 week pre-chill for the other lot.

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 86% to a 96% across all methods (Table 6). On lot B the total viability ranged from an 80% to a 94% (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. In the case of 15-25C and 20-35C there was higher dormancy as well in the four week pre-chill. This could lead one to believe that the longer pre-chill could be sending the seed into a secondary type dormancy. The 20-30 temperature combination showed the least amount of dormancy remaining at the end of the testing period. 20-30C appeared to provide the best germination temperature with 20-35C close. These two temperature regimes along with a two week pre-chill were recommended to be sent out for comparison amongst labs to determine if 20-30C is significantly better.

Six of the seven laboratories returned results. Lab 3 did not complete the 20-30C method. Lab 2 stated that their samples were 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, second, final counts, and total viable than the proposed method of 20-35C. The dormant, abnormal, and dead were significantly higher at the proposed method of 20-35C (Table 8). The ANOVA data for total viability appears in Table 23.

Tables 9-15 show the germination results among the laboratories for the proposed method of 20-35C with a two week pre-chill for lots a, b, c, d, e, f, and g. Lab two had significantly higher dormancy due to the replicates begin to dry out. Lab one and five tended to have higher abnormal than the other laboratories.

Tables 16-22 show the germination results among the laboratories for the proposed method of 20-30C with a two week pre-chill for lots a, b, c, d, e, f, and g. Lab three did not test this method. Again lab one and lab five tended to have higher abnormal than the other laboratories.

The variability among labs is more than the variability among methods. Across all laboratories and all samples the 20-30C had significantly higher germination results. The average increase in total viable for 20-30C was 7% over the 20-35C method. Seed being sold off a pure live seed basis this can make a significant difference to the seed company.

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Appendix

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

15-25 C	1st count (7 days)	2nd count (14 days)	3rd count (21 days)	Final count (28 days)	Total Germ	abs	dead	dormant
1 week prechill 15-25								
a	62	20	4	0	86	6	6	2
b	68	21	2	0	91	1	8	0
2 week prechill 15-25								
a	3	82	3	0	88	5	7	0
b	17	73	0	0	90	2	8	0
4 week prechill 15-25								
a	75	9	0	0	84	8	5	3
b	90	0	0	0	90	0	9	1

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

10-30C	1st count (7 days)	2nd count (14 days)	3rd count (21 days)	Final count (28 days)	Total Germ	abs	dead	dormant
1 week prechill 10-30								
a	0	62	16	0	78	6	9	7
b	0	66	8	1	75	5	12	8
2 week prechill 10-30								
a	0	62	12	2	76	8	2	14
b	0	68	5	1	74	3	17	6
4 week prechill 10-30								
a	42	35	0	0	77	4	7	12
b	65	19	0	0	84	4	8	4

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

20-30C	1st count (7days)	2nd count (14 days)	3rd count (21 days)	Final count (28 days)	Total Germ	abs	dead	dormant
1 week prechill 20-30								
a	83	0	6	0	89	5	4	2
b	84	1	0	0	85	3	12	0
2 week prechill 20-30								
a	84	8	0	0	92	5	3	0
b	87	3	0	0	90	0	10	0
4 week prechill 20-30								
a	93	0	0	0	93	3	4	0
b	94	0	0	0	94	0	6	0

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

sample	1st count (7 days)	2nd count (14 days)	3rd count (21 days)	Final count (28 days)	Total Germ	abs	dead	dormant
1 week prechill 20-35								
a	38	26	0	0	64	11	9	17
b	36	20	16	0	72	10	8	10
2 week prechill 20-35								
a	73	23	0	0	96	1	3	0
b	48	42	2	0	92	7	0	1
4 week prechill 20-35								
a	78	8	0	0	86	4	6	4
b	76	12	0	0	88	1	9	2

Table 5. Average germination, abnormal, dead, dormant and total viable for each in-house regime with a 3-5C prechill.

	Germ	Abs	Dead	Dormant	Viable
A 15-25 1 week prechill	86	6	6	2	88
B 15-25 1 week prechill	91	1	8	0	91
A 15-25 2 week prechill	88	5	7	0	88
B 15-25 2 week prechill	90	2	8	0	90
A 15-25 4 week prechill	84	8	5	3	87
B 15-25 4 week prechill	90	0	9	1	91
A 10-30 1 week prechill	79	6	9	7	86
B 10-30 1 week prechill	75	5	12	8	83
A 10-30 2 week prechill	76	8	2	14	90
B 10-30 2 week prechill	74	3	17	6	80
A 10-30 4 week prechill	77	4	7	12	89
B 10-30 4 week prechill	84	4	8	4	88
A 20-30 1 week prechill	89	5	4	2	91
B 20-30 1 week prechill	85	3	12	0	85
A 20-30 2 week prechill	89	5	4	2	91
B 20-30 2 week prechill	85	3	12	0	85
A 20-30 4 week prechill	92	5	3	0	92
B 20-30 4 week prechill	94	0	6	0	94
A 20-35 1 week prechill	74	11	9	17	91
B 20-35 1 week prechill	72	10	8	10	82
A 20-35 2 week prechill	96	1	3	0	96
B 20-35 2 week prechill	86	13	0	1	87
A 20-35 4 week prechill	86	4	6	4	90
B 20-35 4 week prechill	88	1	9	2	90

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

	Germ	Abs	Dead	Dormant	Viable
A 20-35 2 week prechill	96	1	3	0	96
A 20-30 4 week prechill	92	5	3	0	92
A 20-30 1 week prechill	89	5	4	2	91
A 20-30 2 week prechill	89	5	4	2	91
A 20-35 1 week prechill	74	11	9	17	91
A 10-30 2 week prechill	76	8	2	14	90
A 20-35 4 week prechill	86	4	6	4	90
A 10-30 4 week prechill	77	4	7	12	89
A 15-25 1 week prechill	86	6	6	2	88
A 15-25 2 week prechill	88	5	7	0	88
A 15-25 4 week prechill	84	8	5	3	87
A 10-30 1 week prechill	79	6	9	7	86

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

	Germ	Abs	Dead	Dormant	Viable
B 20-30 4 week prechill	94	0	6	0	94
B 20-35 2 week prechill	92	7	0	1	93
B 15-25 1 week prechill	91	1	8	0	91
B 15-25 4 week prechill	90	0	9	1	91
B 15-25 2 week prechill	90	2	8	0	90
B 20-35 4 week prechill	88	1	9	2	90
B 20-30 2 week prechill	90	0	10	0	90
B 10-30 4 week prechill	84	4	8	4	88
B 20-30 1 week prechill	85	3	12	0	85
B 10-30 1 week prechill	75	5	12	8	83
B 20-35 1 week prechill	72	10	8	10	82
B 10-30 2 week prechill	74	3	17	6	80

Table 8. Statistical analysis of the proposed method #1 20-35C and proposed method #2 20-30C

	Method 1 (20-35C)	Method 2 (20-30C)		
1st count	43.03a	59.29b	P=0.0000	LSD (0.05) = 0.9187
2nd count	69.55a	81.91b	P=0.0000	LSD (0.05) = 0.9647
Final count	75.32a	86.25b	P=0.0000	LSD (0.05) = 0.9821
Abs	7.44a	5.44b		
Deads	8.11a	6.48b		
Dormant	8.75a	1.96b		
Total Viable	84.07a	88.21b	P=0.0000	LSD (0.05) = 0.8477

Table 9. Germination results for lot a with proposed method of 20-35C with 2 week pre-chill 3-5C

Lot a	normal	abs	dead	dormant	total viable
LAB 1	52	27	4	17	69
LAB 2	45	0	5	50	95
LAB 3	95	0	3	2	97
LAB 4	79	10	6	5	84
LAB 5	72	14	13	1	73
LAB 6	65	24	11	0	65
Max	95	27	13	50	97
Min	45	0	3	0	65
Average	68	12.5	7	12.5	80.5
STDV	18.220867	11.519549	4.0496913	19.398454	13.590438

Table 10. Germination results for lot b with proposed method of 20-35C with 2 week pre-chill 3-5C

Lot b	normal	abs	dead	dormant	total viable
LAB 1	62	20	14	4	66
LAB 2	69	0	4	27	96
LAB 3	94	0	5	1	95
LAB 4	85	5	9	1	86
LAB 5	71	16	13	0	71
LAB 6	91	0	9	0	91
Max	94	20	14	27	96
Min	62	0	4	0	66
Average	78.666667	6.8333333	9	5.5	84.166667
STDV	13.094528	8.953584	4.0496913	10.634848	12.734468

Table 11. Germination results for lot c with proposed method of 20-35C with 2 week pre-chill 3-5C

Lot c	normal	abs	dead	dormant	total viable
LAB 1	57	14	8	21	78
LAB 2	71	0	1	28	99
LAB 3	94	0	5	1	95
LAB 4	90	4	3	3	93
LAB 5	77	10	13	0	77
LAB 6	90	1	9	0	90
Max	94	14	13	28	99
Min	57	0	1	0	77
Average	79.833333	4.8333333	6.5	8.8333333	88.666667
STDV	14.246637	5.8793424	4.3703547	12.38413	9.1360093

Table 12. Germination results for lot d with proposed method of 20-35C with 2 week pre-chill 3-5C

Lot d	normal	abs	dead	dormant	total viable
LAB 1	63	15	9	13	76
LAB 2	60	0	4	36	96
LAB 3	91	0	7	2	93
LAB 4	92	1	4	3	95
LAB 5	73	12	15	0	73
LAB 6	90	2	8	0	90
Max	92	15	15	36	96
Min	60	0	4	0	73
Average	78.166667	5	7.83333333	9	87.166667
STDV	14.716204	6.6932802	4.070217	14.085453	10.068101

Table 13. Germination results for lot e with proposed method of 20-35C with 2 week pre-chill 3-5C

Lot e	normal	abs	dead	dormant	total viable
LAB 1	52	27	4	17	69
LAB 2	66	0	1	33	99
LAB 3	94	0	2	4	98
LAB 4	96	0	3	1	97
LAB 5	86	7	7	0	86
LAB 6	94	3	3	0	94
Max	96	7	7	33	99
Min	66	0	1	0	86
Average	87.2	2	3.2	7.6	94.8
STDV	12.457929	3.082207	2.2803509	14.293355	5.2630789

Table 14. Germination results for lot f with proposed method of 20-35C with 2 week pre-chill 3-5C

Lot f	normal	abs	dead	dormant	total viable
LAB 1	60	18	13	9	69
LAB 2	71	0	9	20	91
LAB 3	85	0	13	2	87
LAB 4	79	4	12	5	84
LAB 5	64	18	18	0	64
LAB 6	86	1	13	0	86
Max	86	18	18	20	91
Min	64	0	9	0	64
Average	77	4.6	13	5.4	82.4
STDV	9.4074439	7.6681158	3.2403703	8.4142736	10.597169

Table 15. Germination results for lot g with proposed method of 20-35C with 2 week pre-chill 3-5C

Lot g	normal	abs	dead	dormant	total viable
LAB 1	68	13	6	13	81
LAB 2	90	0	3	7	97
LAB 3	95	0	3	2	97
LAB 4	89	3	4	4	93
LAB 5	76	11	12	1	77
LAB 6	90	1	9	0	90
Max	95	11	12	7	97
Min	76	0	3	0	77
Average	88	3	6.2	2.8	90.8
STDV	7.1063352	4.6368092	4.0865633	2.7748874	8.2583291

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

Lot a	normal	abs	dead	dormant	total viable
LAB 1	55	35	10	0	55
LAB 2	86	0	3	11	97
LAB 4	82	13	5	0	82
LAB 5	78	11	10	1	79
LAB 6	81	12	7	0	81
Max	86	35	10	11	97
Min	55	0	3	0	55
Average	76.4	14.2	7	2.4	78.8
STDV	12.300406	12.755391	3.082207	4.8270074	15.10629

Table 17. Germination results for lot b with proposed method 20-30C with 2 week pre-chill 3-5C

Lot b	normal	abs	dead	dormant	total viable
LAB 1	81	11	8	0	81
LAB 2	94	0	3	3	97
LAB 4	91	0	9	0	91
LAB 5	86	5	9	0	86
LAB 6	92	2	6	0	92
Max	94	11	9	3	97
Min	81	0	3	0	81
Average	88.8	3.6	7	0.6	89.4
STDV	5.2630789	4.6151923	2.5495098	1.3416408	6.1073726

Table 18. Germination results for lot c with proposed method 20-30C with 2 week pre-chill 3-5C

Lot c	normal	abs	dead	dormant	total viable
LAB 1	85	10	5	0	85
LAB 2	96	0	1	3	99
LAB 4	93	0	7	0	93
LAB 5	90	5	5	0	90
LAB 6	96	1	3	0	96
Max	96	10	7	3	99
Min	85	0	1	0	85
Average	92	3.2	4.2	0.6	92.6
STDV	4.6368092	4.3243497	2.2803509	1.3416408	5.4129474

Table 19. Germination results for lot d with proposed method 20-30C with 2 week pre-chill 3-5C

Lot d	normal	abs	dead	dormant	total viable
LAB 1	78	15	7	0	78
LAB 2	92	0	6	2	94
LAB 4	95	0	5	0	95
LAB 5	89	5	6	0	89
LAB 6	93	1	6	0	93
Max	95	15	7	2	95
Min	78	0	5	0	78
Average	89.4	4.2	6	0.4	89.8
STDV	6.7305275	6.3796552	0.7071068	0.8944272	6.9785385

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

Lot e	normal	abs	dead	dormant	total viable
LAB 1	81	17	2	0	81
LAB 2	97	0	1	2	99
LAB 4	95	1	5	0	95
LAB 5	93	4	3	0	93
LAB 6	96	2	2	0	96
Max	97	4	5	2	99
Min	93	0	1	0	93
Average	95.25	1.75	2.75	0.5	95.75
STDV	1.7078251	1.7078251	1.7078251	1	2.5

Table 21. Germination results for lot f with proposed method 20-30C with 2 week pre-chill 3-5C

Lot f	normal	abs	dead	dormant	total viable
LAB 1	72	11	17	0	72
LAB 2	91	0	4	5	96
LAB 4	87	2	9	2	89
LAB 5	75	9	16	0	75
LAB 6	86	2	12	0	86
Max	91	9	16	5	96
Min	75	0	4	0	75
Average	84.75	3.25	10.25	1.75	86.5
STDV	6.8495742	3.9475731	5.057997	2.3629078	8.7368949

Table 22. Germination results for lot g with proposed method 20-30C with 2 week pre-chill 3-5C

Lot g	normal	abs	dead	dormant	total viable
LAB 1	84	10	6	0	84
LAB 2	56	0	3	41	97
LAB 4	94	0	6	0	94
LAB 5	89	5	5	1	90
LAB 6	95	1	4	0	95
Max	95	5	6	41	97
Min	56	0	3	0	90
Average	83.5	1.5	4.5	10.5	94
STDV	18.520259	2.3804761	1.2909944	20.338797	2.9439203

Table 23. Analysis of variance for the total viability of seeds and lab-to-lab variation on the germination of seven milkweed samples tested at 5 laboratories using the same germination parameters

SOURCE	DF	S.S	M.S	F VALUE	P VALUE
LOTS	6	50004.00	834.00	64.43	0.0000
METHOD	1	717.81	717.81	55.46	0.0000
LOT X METHOD	6	190.28	31.71	2.45	0.0261
LAB	4	13447.00	3361.80	259.73	0.0000
LOT X LAB	24	1304.00	54.33	4.20	0.0000
METHOD X LAB	4	652.08	163.02	12.59	0.0000
LOT X METHOD X LAB	24	676.55	28.19	2.18	0.0019