**Rule Change Proposal 9**

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

**Present Rule:** New Rule

**Proposed Rule:**

| Kind of Seed | Substrate[a] | Temperature (C°) | First Count (days) | Final Count (days) | Specific requirements | Fresh and Dormant |
|---|---|---|---|---|---|---|
| *Asclepias incarnata* L., swamp milkweed | P | 20-30; 15-25 | 7 | 21 | $KNO_3$ Prechill 14 days at 3-5C. Ungerminated seeds: see sec. 6.2f and 6.9m | |

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

**Supporting Evidence:**

Materials and methods:

Three lots of *Asclepias incarnata* 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.

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 the lots out for a lab referee. The in-house experiment showed that three methods appeared to be like one another due to the low quality of one of the lots.

Seven labs were then sent three lots of seed to test using three different methods. Method 1 was a one week pre-chill and then to germinate at 20-35C for 21 days. Method 2 was a two week pre-chill and then to germinate at 15-25C for 21 days. Method 3 was a two week pre-chill and then to germinate at 20-30C for 21 days. Laboratories were asked to do a 7, 14, and 21 day count on each replicate. 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 not significant growth between 21 to 28 days. The final count days are recommended to be 21 days. No matter the germination temperature the growth was much slower with a one week pre-chill. At the 15-25C germination temperature (Table 1), the best germination was obtained with a

two week pre-chill for lot a and a four week pre-chill for lot b. At the 10-30C germination temperature (Table 2), the best growth was with a 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. The best growth was reached with a two week pre-chill. At the 20-35C germination temperature (Table 4), the two week pre-chill produced the best germination.

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 7% to a 26% across all methods (Table 6). On lot B the total viability ranged from a 69% to an 88% (Table 7). Lot had a significant amount of fungal growth and a lot of decay. This made it difficult to determine what would accurately be a better temperature regime. Therefore the 15-25C, 20-30C and 20-35C with a two week pre-chill were recommended to be sent to the laboratories for the referee.

Six of the seven laboratories returned results. Lab 2 only completed method 1. Lab 3 did not complete method 2. Lab 5 did not complete method 3. Lab 3 was significantly higher in dormant than other laboratories on some of the lots due to the test conditions lacking moisture. This along with the low germination of two of the lots made the statistics more of a challenge. The complete statistical analysis was only ran on the three laboratories that completed all three methods (Table 8). Lots b and c were labelled to be low in germination and high in dormancy. All laboratories found this two lots to be low in germination and that the remaining seeds were dead and completely covered in fungus. The 15-25C and the 20-30C method statically are not significantly different on the total viable.

Tables 9-11 show the results for the six laboratories using the one week pre-chill 21 day germ test at 20-35C testing method for lots a, b, and c. The ranges are very close together across all three lots when removing the high dormancy of laboratory three on lot a.

Tables 12-14 show the results for the four laboratories using the two week pre-chill 21 day germ test at 15-25C testing methods for lots a, b, and c. The ranges are very close together across all three lots.

Tables 15-17 show the test results for the four laboratories using the two week pre-chill 21 day germ test at 20-30C testing methods for lots a, b, and c. The ranges are very close together across all three lots.

For lot a method two at 15-25C with a two week pre-chill showed the highest average germination results. Method three at 20-30C showed the highest average total viable for lot a as well.

For lot b method three at 20-30C showed the highest average germination along with the highest average total viable.

For lot c method three at 20-30C showed the highest average germination. Method two at 15-25C showed the highest average total viable.

Based off the statistics ran, it showed that the 15-25C and 20-30C method were not significantly different from one another. Also based off the germination results it appeared that the 15-25C and 20-30C method produced very similar results that are within tolerance of one another. Therefore both temperature regime/methods are being recommended for this species. The variability among labs was greater than the variability among methods.

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Appendix

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

| sample | 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** | 2 | 4 | 0 | 0 | 6 | 4 | 82 | 8 |
| **b** | 5 | 55 | 2 | 0 | 62 | 2 | 26 | 10 |
| **2 week prechill 15-25** | | | | | | | | |
| **a** | 9 | 2 | 0 | 0 | 11 | 3 | 71 | 15 |
| **b** | 62 | 12 | 0 | 0 | 74 | 2 | 23 | 3 |
| **4 week prechill 15-25** | | | | | | | | |
| **a** | 11 | 0 | 0 | 0 | 11 | 3 | 76 | 10 |
| **b** | 64 | 10 | 0 | 0 | 74 | 4 | 14 | 8 |

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

| sample | 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 | 12 | 1 | 0 | 13 | 1 | 80 | 6 |
| **b** | 8 | 40 | 2 | 0 | 50 | 1 | 30 | 20 |
| **2 week prechill 10-30** | | | | | | | | |
| **a** | 9 | 1 | 0 | 0 | 10 | 3 | 79 | 8 |
| **b** | 15 | 38 | 0 | 0 | 53 | 2 | 25 | 20 |
| **4 week prechill 10-30** | | | | | | | | |
| **a** | 12 | 0 | 0 | 0 | 12 | 2 | 79 | 7 |
| **b** | 10 | 44 | 0 | 0 | 54 | 2 | 29 | 15 |

Table 3. Germination counts for the 2 lots tested in-house at 20-30C with 3-5C prechill

| sample | 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** | 14 | 2 | 0 | 0 | 16 | 1 | 82 | 1 |
| **b** | 42 | 20 | 10 | 0 | 72 | 2 | 14 | 12 |
| **2 week prechill 20-30** | | | | | | | | |
| **a** | 11 | 1 | 0 | 0 | 12 | 2 | 85 | 1 |
| **b** | 58 | 25 | 0 | 0 | 83 | 0 | 12 | 5 |
| **4 week prechill 20-30** | | | | | | | | |
| **a** | 12 | 0 | 0 | 0 | 12 | 4 | 80 | 4 |
| **b** | 50 | 20 | 5 | 0 | 75 | 2 | 14 | 9 |

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

| sample | 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** | | | | | | | | |
| **a** | 14 | 0 | 0 | 0 | 14 | 3 | 79 | 4 |
| **b** | 24 | 50 | 0 | 0 | 74 | 2 | 18 | 6 |
| **2 week prechill 20-35** | | | | | | | | |
| **a** | 5 | 0 | 0 | 0 | 5 | 3 | 90 | 2 |
| **b** | 79 | 5 | 0 | 0 | 84 | 1 | 13 | 2 |
| **4 week prechill 20-35** | | | | | | | | |
| **a** | 8 | 0 | 0 | 0 | 8 | 6 | 74 | 12 |
| **b** | 60 | 20 | 0 | 0 | 80 | 5 | 11 | 4 |

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** | 6 | 4 | 82 | 8 | 14 |
| **B 15-25 1 week prechill** | 62 | 2 | 26 | 10 | 72 |
| **A 15-25 2 week prechill** | 11 | 3 | 71 | 15 | 26 |
| **B 15-25 2 week prechill** | 74 | 2 | 23 | 3 | 77 |
| **A 15-25 4 week prechill** | 11 | 3 | 76 | 10 | 21 |
| **B 15-25 4 week prechill** | 74 | 4 | 14 | 8 | 82 |
| **A 10-30 1 week prechill** | 13 | 1 | 80 | 6 | 19 |
| **B 10-30 1 week prechill** | 50 | 1 | 30 | 20 | 70 |
| **A 10-30 2 week prechill** | 10 | 3 | 79 | 8 | 18 |
| **B 10-30 2 week prechill** | 53 | 2 | 25 | 20 | 73 |
| **A 10-30 4 week prechill** | 12 | 2 | 79 | 7 | 19 |
| **B 10-30 4 week prechill** | 54 | 2 | 29 | 15 | 69 |
| **A 20-30 1 week prechill** | 16 | 1 | 82 | 1 | 17 |
| **B 20-30 1 week prechill** | 72 | 2 | 14 | 12 | 84 |
| **A 20-30 2 week prechill** | 12 | 2 | 85 | 1 | 13 |
| **B 20-30 2 week prechill** | 83 | 0 | 12 | 5 | 88 |
| **A 20-30 4 week prechill** | 12 | 4 | 80 | 4 | 16 |
| **B 20-30 4 week prechill** | 75 | 2 | 14 | 9 | 84 |
| **A 20-35 1 week prechill** | 14 | 3 | 79 | 4 | 18 |
| **B 20-35 1 week prechill** | 74 | 2 | 18 | 6 | 80 |
| **A 20-35 2 week prechill** | 5 | 3 | 90 | 2 | 7 |
| **B 20-35 2 week prechill** | 84 | 1 | 13 | 2 | 86 |
| **A 20-35 4 week prechill** | 8 | 6 | 74 | 12 | 20 |
| **B 20-35 4 week prechill** | 80 | 5 | 11 | 4 | 84 |

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

| | Germ | Abs | Dead | Dormant | Viable |
|---|---|---|---|---|---|
| **A 15-25 2 week prechill** | 11 | 3 | 71 | 15 | 26 |
| **A 15-25 4 week prechill** | 11 | 3 | 76 | 10 | 21 |
| **A 20-35 4 week prechill** | 8 | 6 | 74 | 12 | 20 |
| **A 10-30 1 week prechill** | 13 | 1 | 80 | 6 | 19 |
| **A 10-30 4 week prechill** | 12 | 2 | 79 | 7 | 19 |
| **A 10-30 2 week prechill** | 10 | 3 | 79 | 8 | 18 |
| **A 20-35 1 week prechill** | 14 | 3 | 79 | 4 | 18 |
| **A 20-30 1 week prechill** | 16 | 1 | 82 | 1 | 17 |
| **A 20-30 4 week prechill** | 12 | 4 | 80 | 4 | 16 |
| **A 15-25 1 week prechill** | 6 | 4 | 82 | 8 | 14 |
| **A 20-30 2 week prechill** | 12 | 2 | 85 | 1 | 13 |
| **A 20-35 2 week prechill** | 5 | 3 | 90 | 2 | 7 |

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

| | Germ | Abs | Dead | Dormant | Viable |
|---|---|---|---|---|---|
| **B 20-30 2 week prechill** | 83 | 0 | 12 | 5 | 88 |
| **B 20-35 2 week prechill** | 84 | 1 | 13 | 2 | 86 |
| **B 20-30 1 week prechill** | 72 | 2 | 14 | 12 | 84 |
| **B 20-30 4 week prechill** | 75 | 2 | 14 | 9 | 84 |
| **B 20-35 4 week prechill** | 80 | 5 | 11 | 4 | 84 |
| **B 15-25 4 week prechill** | 74 | 4 | 14 | 8 | 82 |
| **B 20-35 1 week prechill** | 74 | 2 | 18 | 6 | 80 |
| **B 15-25 2 week prechill** | 74 | 2 | 23 | 3 | 77 |
| **B 10-30 2 week prechill** | 53 | 2 | 25 | 20 | 73 |
| **B 15-25 1 week prechill** | 62 | 2 | 26 | 10 | 72 |
| **B 10-30 1 week prechill** | 50 | 1 | 30 | 20 | 70 |
| **B 10-30 4 week prechill** | 54 | 2 | 29 | 15 | 69 |

Table 8. Statistical analysis of the three proposed methods.

| Method | 1ST COUNT | 2nd count | FINAL COUNT | ABS | DEAD | DORMANT | Total Viable | 1ST COUNT | 2ND COUNT | FINAL COUNT | Total Viable |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Method 1 (20-35C) | 17.61 | 30.31 | 32.00 | 3.19 | 63.19 | 1.64 | 33.64 | B | B | NS | A |
| Method 2 (15-25C) | 9.14 | 27.53 | 32.42 | 3.64 | 60.89 | 3.06 | 35.47 | A | A | NS | B |
| Method 3 (20-30C) | 24.44 | 31.39 | 32.83 | 1.806 | 62.42 | 2.889 | 35.72 | C | C | NS | B |
| | | | | | | | | P=0.0000 | P=0.0000 | P=0.0773 | P=0.0040 |
| | | | | | | | | LSD (0.05) = 1.177 | LSD (0.05) = 1.271 | LSD (0.05) = 1.292 | LSD (0.05) = 1.312 |

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

| Lot a | normal | abs | dead | dormant | total viable |
|---|---|---|---|---|---|
| **LAB 1** | 78 | 2 | 16 | 4 | 82 |
| **LAB 2** | 78 | 0 | 12 | 10 | 88 |
| **LAB 3** | 37 | 0 | 18 | 45 | 82 |
| **LAB 4** | 78 | 4 | 18 | 0 | 78 |
| **LAB 5** | 89 | 1 | 10 | 0 | 89 |
| **LAB 7** | 88 | 1 | 10 | 1 | 89 |
| **Max** | 89 | 4 | 18 | 45 | 89 |
| **Min** | 37 | 0 | 10 | 0 | 78 |
| **Average** | 74.6666667 | 1.33333333 | 14 | 10 | 84.6666667 |
| **STDV** | 19.158984 | 1.50554531 | 3.79473319 | 17.5613211 | 4.63321343 |

Table 10. Germination results for Lot b with the proposed method of 20-35C with a one week 3-5C pre-chill

| Lot b | normal | abs | dead | dormant | total viable |
|---|---|---|---|---|---|
| **LAB 1** | 5 | 6 | 87 | 2 | 7 |
| **LAB 2** | 7 | 0 | 90 | 3 | 10 |
| **LAB 3** | 7 | 0 | 88 | 5 | 12 |
| **LAB 4** | 4 | 2 | 94 | 0 | 4 |
| **LAB 5** | 6 | 1 | 93 | 0 | 6 |
| **LAB 7** | 5 | 7 | 88 | 0 | 5 |
| **Max** | 7 | 7 | 94 | 5 | 12 |
| **Min** | 4 | 0 | 87 | 0 | 4 |
| **Average** | 5.66666667 | 2.66666667 | 90 | 1.66666667 | 7.33333333 |
| **STDV** | 1.21106014 | 3.07679487 | 2.89827535 | 2.06559112 | 3.07679487 |

Table 11. Germination results for Lot C with the proposed method of 20-35C with a one week 3-5C pre-chill

| Lot c | normal | abs | dead | dormant | total viable |
|---|---|---|---|---|---|
| **LAB 1** | 11 | 4 | 77 | 8 | 19 |
| **LAB 2** | 15 | 0 | 84 | 1 | 16 |
| **LAB 3** | 12 | 0 | 85 | 3 | 15 |
| **LAB 4** | 8 | 3 | 89 | 0 | 8 |
| **LAB 5** | 8 | 3 | 89 | 0 | 8 |
| **LAB 7** | 13 | 1 | 85 | 1 | 14 |
| **Max** | 15 | 4 | 89 | 8 | 19 |
| **Min** | 8 | 0 | 77 | 0 | 8 |
| **Average** | 11.1666667 | 1.83333333 | 84.8333333 | 2.16666667 | 13.3333333 |
| **STDV** | 2.786874 | 1.72240142 | 4.40075751 | 3.06050105 | 4.45720391 |

Table 12. Germination results for Lot A with the proposed method of 15-25C with a two week 3-5C pre-chill

| Lot a | normal | abs | dead | dormant | total viable |
|---|---|---|---|---|---|
| **LAB 1** | 82 | 1 | 14 | 3 | 85 |
| **LAB 4** | 79 | 3 | 18 | 0 | 79 |
| **LAB 5** | 86 | 0 | 14 | 0 | 86 |
| **LAB 7** | 81 | 3 | 12 | 4 | 85 |
| **Max** | 86 | 3 | 18 | 4 | 86 |
| **Min** | 79 | 0 | 12 | 0 | 79 |
| **Average** | 82 | 1.75 | 14.5 | 1.75 | 83.75 |
| **STDV** | 2.94392029 | 1.5 | 2.51661148 | 2.06155281 | 3.20156212 |

Table 13. Germination results for Lot b with the proposed method of 15-25C with a two week 3-5C pre-chill

| Lot b | normal | abs | dead | dormant | total viable |
|---|---|---|---|---|---|
| LAB 1 | 6 | 6 | 84 | 4 | 10 |
| LAB 4 | 7 | 4 | 89 | 0 | 7 |
| LAB 5 | 5 | 5 | 90 | 0 | 5 |
| LAB 7 | 7 | 5 | 88 | 0 | 7 |
| Max | 7 | 6 | 90 | 4 | 10 |
| Min | 5 | 4 | 84 | 0 | 5 |
| Average | 6.25 | 5 | 87.75 | 1 | 7.25 |
| STDV | 0.95742711 | 0.81649658 | 2.62995564 | 2 | 2.06155281 |

Table 14. Germination results for Lot c with the proposed method of 15-25C with a two week 3-5C pre-chill

| Lot c | normal | abs | dead | dormant | total viable |
|---|---|---|---|---|---|
| LAB 1 | 11 | 4 | 68 | 17 | 28 |
| LAB 4 | 12 | 4 | 84 | 0 | 12 |
| LAB 5 | 14 | 3 | 83 | 0 | 14 |
| LAB 7 | 8 | 3 | 89 | 0 | 8 |
| Max | 14 | 4 | 89 | 17 | 28 |
| Min | 8 | 3 | 68 | 0 | 8 |
| Average | 11.25 | 3.5 | 81 | 4.25 | 15.5 |
| STDV | 2.5 | 0.57735027 | 9.05538514 | 8.5 | 8.6986589 |

Table 15. Germination results for Lot a with the proposed method of 20-30C with a two week 3-5C pre-chill

| Lot a | normal | abs | dead | dormant | total viable |
|---|---|---|---|---|---|
| LAB 1 | 74 | 1 | 17 | 8 | 82 |
| LAB 3 | 71 | 0 | 12 | 15 | 86 |
| LAB 4 | 87 | 2 | 11 | 0 | 87 |
| LAB 7 | 70 | 1 | 16 | 13 | 83 |
| Max | 87 | 2 | 16 | 15 | 87 |
| Min | 70 | 0 | 11 | 0 | 83 |
| Average | 76 | 1 | 13 | 9.333333333 | 85.33333333 |
| STDV | 9.539392014 | 1 | 2.645751311 | 8.144527815 | 2.081665999 |

Table 16. Germination results for Lot b with the proposed method of 20-30C with a two week 3-5C pre-chill

| Lot b | normal | abs | dead | dormant | total viable |
|---|---|---|---|---|---|
| LAB 1 | 10 | 2 | 87 | 1 | 11 |
| LAB 3 | 14 | 0 | 86 | 0 | 14 |
| LAB 4 | 8 | 2 | 90 | 0 | 8 |
| LAB 7 | 8 | 2 | 90 | 0 | 8 |
| Max | 14 | 2 | 90 | 1 | 14 |
| Min | 8 | 0 | 86 | 0 | 8 |
| Average | 10 | 1.5 | 88.25 | 0.25 | 10.25 |
| STDV | 2.828427125 | 1 | 2.061552813 | 0.5 | 2.872281323 |

Table 17. Germination results for Lot c with the proposed method of 20-30C with a two week 3-5C pre-chill

| Lot c | normal | abs | dead | dormant | total viable |
|---|---|---|---|---|---|
| **LAB 1** | **15** | **3** | **78** | **4** | **19** |
| **LAB 3** | **15** | **0** | **84** | **1** | **16** |
| **LAB 4** | **10** | **3** | **87** | **0** | **10** |
| **LAB 7** | **14** | **2** | **84** | **0** | **14** |
| **Max** | **15** | **3** | **87** | **4** | **19** |
| **Min** | **10** | **0** | **78** | **0** | **10** |
| **Average** | **13.5** | **2** | **83.25** | **1.25** | **14.75** |
| **STDV** | **2.380476143** | **1.414213562** | **3.774917218** | **1.892969449** | **3.774917218** |

Table 18. Analysis of variance for the total viability of seeds and lab-to lab variation on the germination of three milkweed samples tested at 4 laboratories using the same germination parameters

| SOURCE | DF | S.S | M.S | F VALUE | P VALUE |
|---|---|---|---|---|---|
| LOTS | 2 | 54886.00 | 27443.00 | 5.92 | 0.0000 |
| METHOD | 2 | 92.54 | 46.27 | 1.90 | 0.0040 |
| LOT X METHOD | 4 | 59.26 | 14.82 | 23.37 | 0.1196 |
| LAB | 2 | 365.33 | 182.66 | 11.14 | 0.0000 |
| LOT X LAB | 4 | 348.43 | 87.11 | 33.61 | 0.0000 |
| METHOD X LAB | 4 | 134.43 | 163.02 | 24.26 | 0.0034 |
| LOT X METHOD X LAB | 8 | 194.09 | 28.19 | 7.82 | 0.0043 |