Milkweed Purity and Germination Study

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Purpose and Design

- Milkweeds are important food sources for Butterflies
  - Multiple species across ecotypes and environs
- Only Two species are listed in the purity rules
- Only ***A. tuberosa*** was listed in Table 6A.
  - A highly unusual temperature range was listed, and search of previous rules indicate this temperature range was historic
- Seeds were provided anonymously from a number of suppliers across the United States, marks on the samples ranged from 2016-2019
- Because of the high value of the seed and the limited supply, intensive in house testing was conducted prior to the referee to refine the scope
- The referee spanned labs that tested native seeds

Purity Rules

- Pure Seed Definitions
- Chaffy Seed Designation
- Working Weights

- Determined in-house to conserve seed
- Data accompanies the Rule Proposal

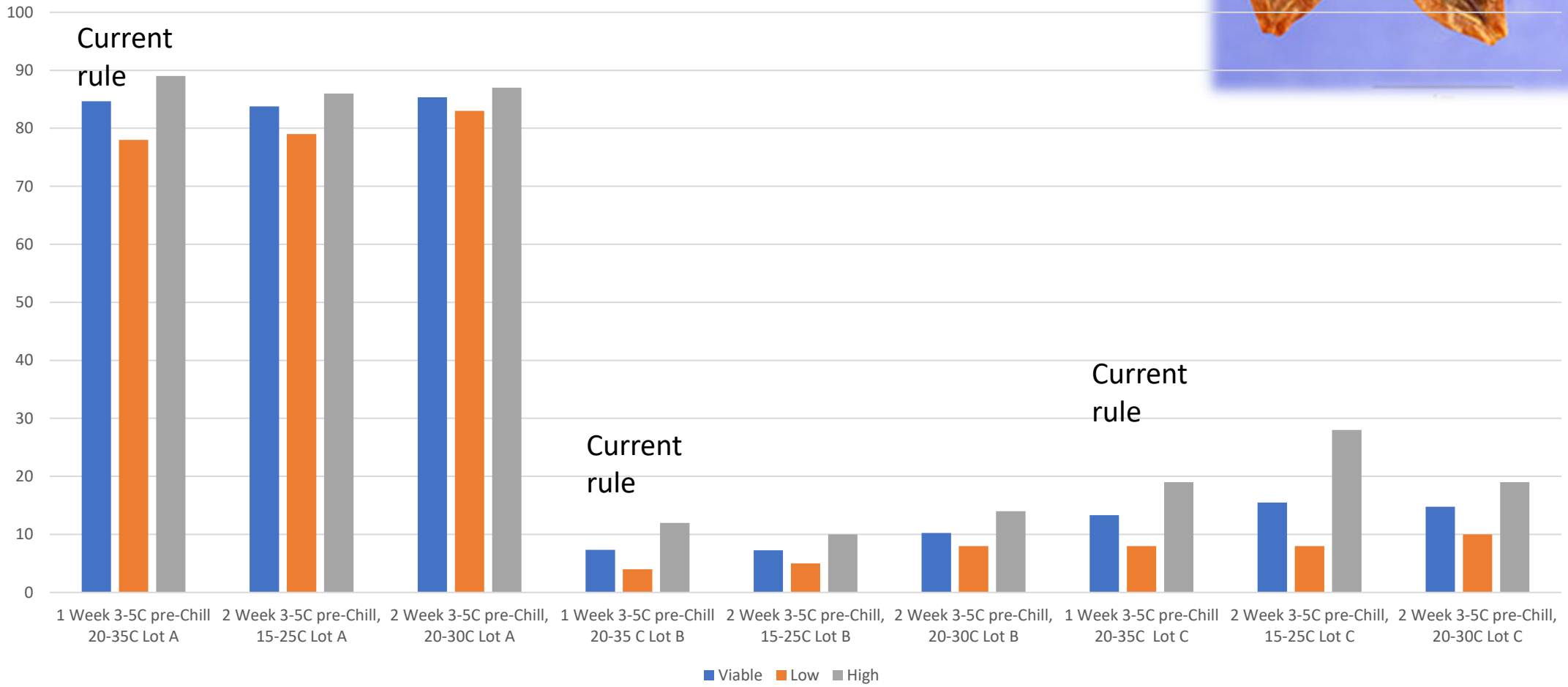
Germination Rules Referee

Proposed Methods Determined in-house to conserve seed

Six to Twelve potential pre-chill geminations were tested per species

- Data accompanies the Rule Proposal
- Participants were requested to only complete the most promising pre-chill and germination temperatures to optimize available resources
- Some participants only had the time and space for one species or some for all species
- Only complete data was included in the following analysis

Asclepias incarnata, swamp milkweed



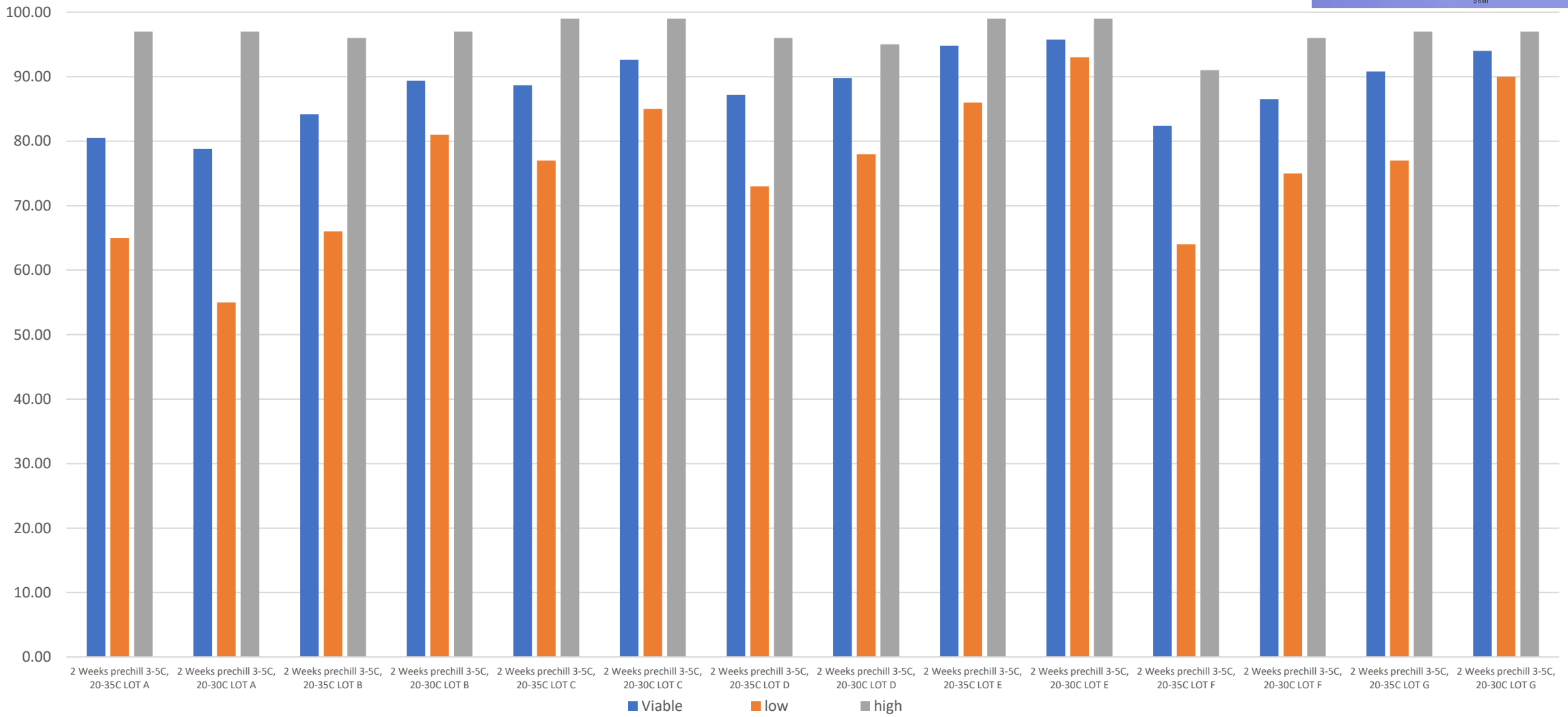
Asclepias incarnata, swamp milkweed

- The two proposed methods were not significantly different
- Lots were significantly different
- Variability between labs was greater than the variability between methods
- The 20-30C method did have the highest average germination across all three lots
- The 20-35C regime (current rule) did not demonstrate a clear superiority, thus labs may conserve temperature regimes by using the two proposed methods.

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.	27443.	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

Asclepius speciosa, Showy milkweed



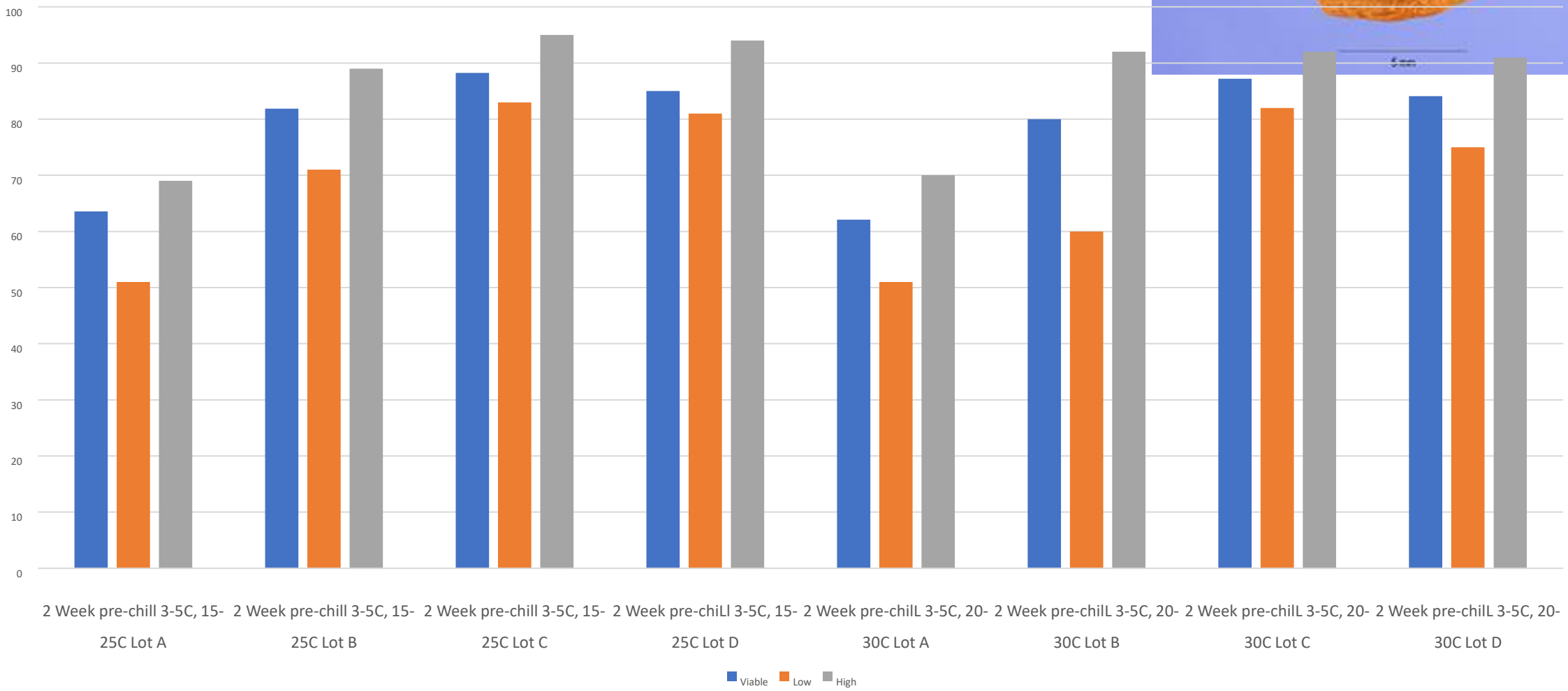
Asclepius speciosa, Showy milkweed

- The proposed method of 20-30C had the highest first, second, final counts and total viability than in this study resulted in an average of 7% higher than the 20-35C method.
- Variability among labs was more than the variability among methods.

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

SOURCE	DF	S.S	M.S	F VALUE	P VALUE
LOTS	6	50004.	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.	3361.8	259.73	0.0000 ***
LOT X LAB	24	1304.0	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

Asclepias syriaca, common milkweed



Asclepias syriaca, common milkweed

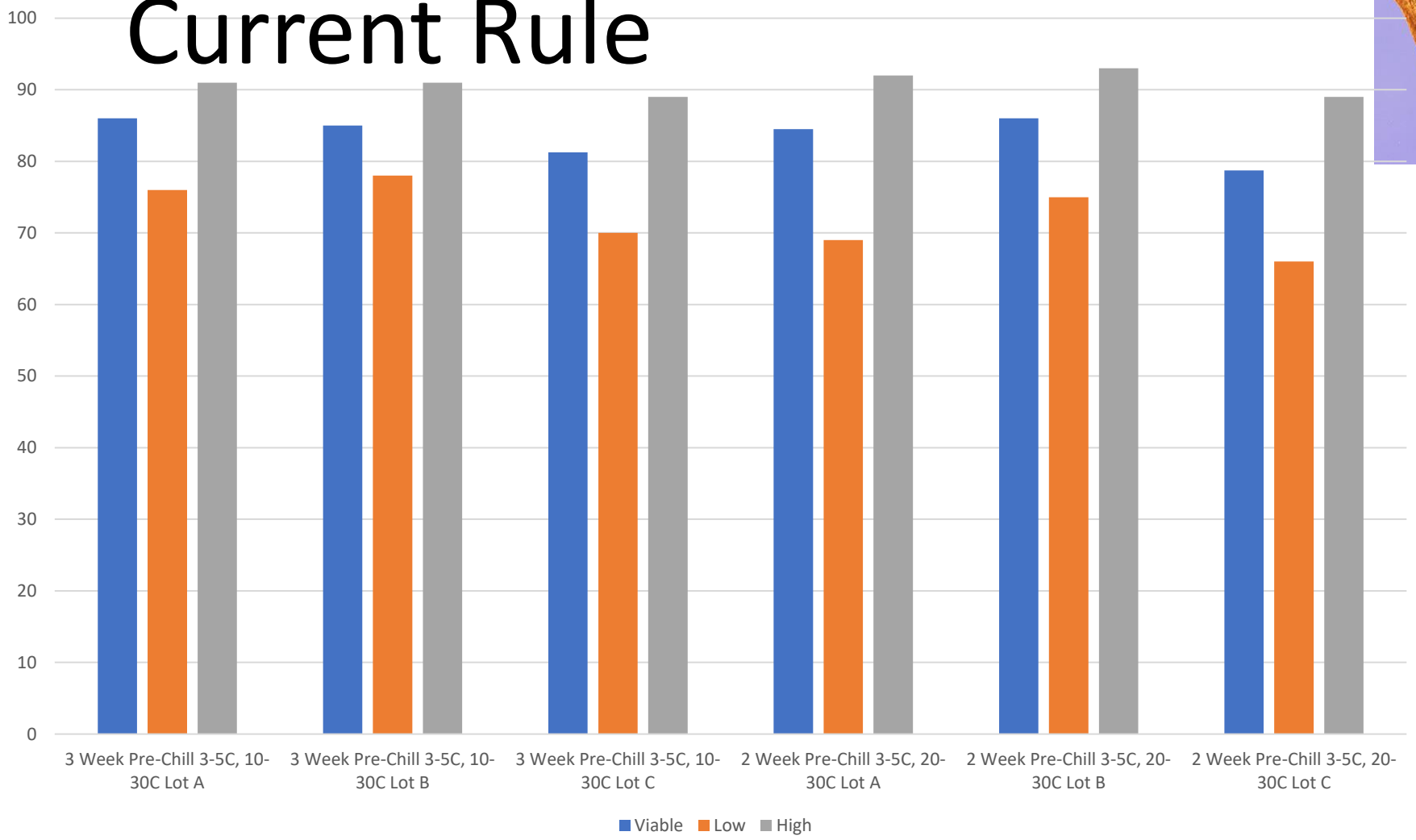
- The two methods were not statistically different.
- The variability between labs was more than the variability between methods.

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	4073.	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.	412.34	50.20	0.0000 ***
LOT X LAB	21	705.9	33.62	4.09	0.0000
METHOD X LAB	7	136.1	19.45	2.37	0.0243
LOT X METHOD X LAB	21	365.8	17.42	2.12	0.0043

Asclepias tuberosa, butterfly milkweed

Current Rule



Asclepias tuberosa, butterfly milkweed

- 20-30C method harmonizes with ISTA rules.
- There was no statistical difference between the current rule and the proposed rule.
- The variability between labs was more than the variability between methods.

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

Conclusions

- Species included in the referee were impacted by the availability of the minimum requirement of att least 3 lots of the same species.
- Pre-screening of potential methods reduced the need for very limited and expensive seed.
  - All data is attached to the Rule proposals
- The Referee utilized only the most promising methods determined by pre-screening.
- Variability between laboratories was often greater than the variability among methods.
- Although some methods were equivalent, the proposed rules were biased toward the method with the highest PLS due to the price of the seed.
- The proposed rules also are impacted by consideration of existing ISTA rules.

Acknowledgements

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