An Assessment of Seed Production and Viability of Putative *Castilleja levisecta* × *C. hispida* Hybrids

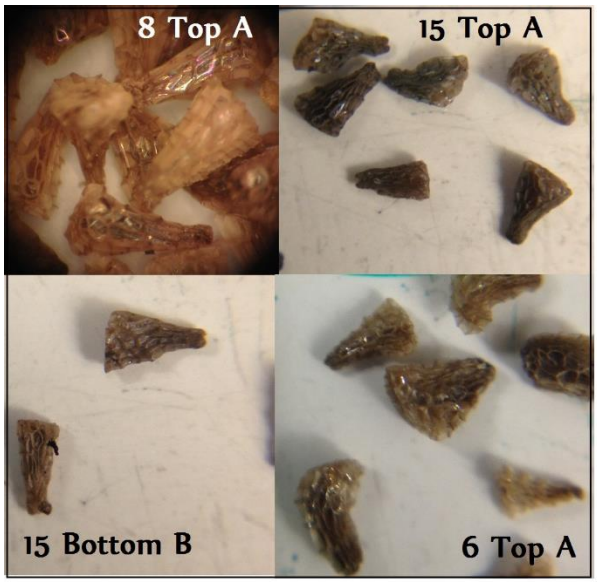

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Table of Contents

Abstract

The objective of this project was to determine seed production and viability of plants sharing morphological traits of *Castilleja levisecta* (CALE) and *C. hispida* (CAHI). Fruiting stems were gathered from 25 putative CALE x CAHI hybrids and from one plant of each species, for a total of 27 maternal plants. Seed production and germination were assessed on a per-capsule basis for 4 capsules per plant, two from the top and two from the bottom of the fruiting stem, for a total of 108 capsules. These data were supplemented with data from a previous experiment testing the effect of host plant identity on seed production in CALE (72 capsules from 36 plants). Hybrid plants produced smaller capsules with less than half as many seeds per capsule as in CALE, though the seeds themselves were larger. Some capsules contained a sizable number of malformed seeds. Capsules from the bottom of the stem had lower seed densities than capsules from the top of the stem. On average, 77% of the seeds in putative hybrids germinated; this rate ranged from 26 to 95% among maternal plants. Germination began after an average of 36 days of stratification, peaked 14 days later, and lasted for 50 days; this timing more closely resembles that of CALE than CAHI. The number of seeds per capsule and the timing of when germination began varied strongly among maternal plants, and may reflect the unique context of individual plants – both their genetic history and their spatial location relative to potential pollen donors of either species. Genetic analyses are underway to determine the hybridization history of these plants. Additional work is required to understand the implications of hybridization for the recovery of CALE.

Background

Castilleja levisecta (CALE), listed federally as a threatened species, is one of the most vulnerable plants in Puget Sound prairies. Historically, CALE occurred from Oregon's Willamette Valley through the western lowlands of Washington to southwestern British Columbia. Most of these populations have been extirpated. Recovery requires the establishment of new populations of the species (USFWS 2000). As a result, CALE has been planted or sown extensively for over ten years in various South Puget Sound prairies.

Another *Castilleja* species, *C. hispida* (CAHI) also occurs in some prairies. CAHI is not endangered and is broadly distributed across low and mid-elevations in the western United States.

Land managers and researchers have known for some time that CALE and CAHI can be intentionally hybridized (Kaye and Blakeley-Smith 2008), but the extent to which they would do so in the field. In the 1990s, one *Castilleja* population on Joint Base Lewis-McChord exhibited characteristics somewhat intermediate between the two species, and it was suggested that it might be of hybrid origin (Mark Egger, personal communication). However, definitive hybrids of the two species were not documented before the greenhouse studies of Kaye and Blakeley-Smith (2008).

The potential for hybridization has increased in recent years because the two species have been sown or planted in close proximity. This is largely because CAHI is a known larval host plant

for Taylor's checkerspot butterfly (*Euphydryas editha taylori*), listed federally as an endangered species, and therefore has been included in Taylor's checkerspot habitat enhancement plantings.

The strongest likelihood of hybridization is when the two species are sown in very close proximity to one another (Clark 2015). This occurred during one phase of the Prairie Habitat Restoration project, which was conducted at two sites in south Puget Sound (Glacial Heritage, West Rocky) and two sites in north Puget Sound (Ebey's Landing, Smith Prairie). However, CAHI establishment was much poorer in north Puget Sound so the likelihood of hybridization is much higher at the south Puget Sound sites.

Hybridization can only occur when pollen is produced on one species at the same time that stigma are receptive on flowers of the other species. However, *Castilleja* flowers are produced throughout the flowering season; when assessed on a fruiting stem, those that were produced first are at the bottom of the stem while those that were produced last are at the top of the stem. Furthermore, CALE begins to flower before CAHI. Together, these observations imply that flowers on the same raceme may have experienced different pollen loads and may differ in hybridization potential. We have speculated, for example, that CALE capsules from the bottom of the stem would be less likely than those from the top of the stem to have hybridized with CAHI. Whether this also applies to putative hybrids is unknown.

Finally, a key unresolved question is whether CALE x CAHI hybrids are sterile or are able to produce viable seeds. By definition, sterile hybrids cannot contribute genes to subsequent generations and thus would be much less of a concern relative to the recovery of CALE. A new project (Kaye 2014) has begun to experimentally assess the viability of hybrids, but it will take several growing seasons to conduct the necessary crosses and backcrosses. In the interim, our project examined putative CALE x CAHI hybrids, of unresolved parentage, to determine whether they produced viable seed.

Project Objective and Benefits

The objective of this project was to determine the seed production and viability of plants sharing morphological traits of CALE and CAHI. Benefits of this project include:

1. Quantifying seed production by putative CALE x CAHI hybrids.
2. Verifying whether the seeds produced by putative hybrids are viable.
3. Exploring whether hybridization potential varies across the flowering season, as reflected by the seed production and seed viability of capsules from the top and bottom of the fruiting stem.

Project Details

Seed Collection

CALE and CAHI plants can usually be distinguished by a few morphological traits (Clark 2015; Figure 1). CALE flowers have yellow-tipped bracts with protruding stigma, while CAHI flowers typically have red or orange-tipped bracts with shorter stigma, though yellow bracts are frequent

in some populations. CALE exhibits a smaller leaf-stem angle than CAHI, and its leaf lobes are shallower. CALE x CAHI hybrids can exhibit an entire spectrum of intermediate lobe and leaf angle traits, with bracts tipped in yellow, orange, or red (Kaye and Blakeley-Smith 2008; Clark 2015).

Plants for this trial were obtained from the Prairie Habitat Restoration project area in the northwest corner of Glacial Heritage Preserve, where some plots were sown with CALE and CAHI in Fall 2009. In late summer of 2014, 25 plants with various intermediate morphologies (Figure 1; Appendix 1) were selected. All of these plants were located in the project's 2010 array. At the time of collection, Peter Dunwiddie assumed they were F1 hybrids based on their intermediate morphology. However, we do not know the hybridization history of these plants: how many generations have occurred in the field, who the parental species were in each generation, etc. One fruiting stem was taken from each plant.

Each fruiting stem contained many more capsules than it was possible to test. However, each capsule could represent a single pollination event and capsules from the same plant can vary in seed quantity and proper seed development, so we selected four capsules per plant, two from the bottom and two from the top of the stem. Each capsule was tracked separately throughout the study.

For comparison, four capsules were also selected from one plant at Glacial Heritage Preserve that exhibited pure CALE morphological traits and one plant at Webster's Native Seed Farm that exhibited pure CAHI traits. In total, therefore, 108 capsules were examined.

Figure 1. Examples of *Castilleja levisecta* (CALE; left), *C. hispida* (CAHI; right), and a putative hybrid (center).

Germination and Viability Testing

Each capsule was weighed, and then the seeds were removed and weighed. A sample of the seeds were photographed (Appendix 1). The seeds were then counted and plated on moistened VWR filter paper in a Petri dish (one per capsule).

Standard germination testing procedures are to expose CALE seeds to 30 days of cold-moist stratification and CAHI seeds to 60-90 days of cold-moist stratification. As advised by CNLM, the putative CALE x CAHI seeds were subjected to the longer 90 day stratification period of CAHI. Petri dishes were placed in a growth chamber simulating winter conditions: a constant temperature of 5°C with 10 hours of light and 14 hours of dark each day. The filter paper was kept moistened throughout the germination test. At the end of this period, seeds were transferred to a growth chamber simulating spring conditions: 16°C and lit for 12 hours and 8°C and dark for 12 hours each day. However, the vast majority of seedlings germinated during winter stratification.

Capsules were processed and entered germination testing in waves to keep the tasks manageable. The first germination tests began on September 23, 2014, and the last began on November 13, 2014. Germination counts were taken up to several times per week. Germinants were removed from the dishes as they were tallied; a subset of these were transplanted into pots in the greenhouse for follow-up study. Testing ended after germination peaked, diminished, and then ceased entirely for at least one week. Germination testing lasted for an average of 12 weeks but continued for up to 20 weeks. The last tallies were made on March 9, 2015.

After the germination test, non-germinating seeds that had not molded to the point of disintegration were tested for viability. Seeds were pierced with a dissecting needle and soaked in a 1% tetrazolium solution for 48 hours at 21°C, as recommended by AOSA (2000). Seeds were checked for viability staining under a dissection scope. Of all the non-germinating seeds tested, only one seed from Plant 5 stained as viable. Therefore, we focus here simply on live seeds and do not distinguish those that germinated from those that did not germinate but were viable.

Data Analysis

Seed mass data and germination data for each capsule are reported in Appendices 2 and 3, respectively. Data were analyzed in two ways: i) comparing hybrids against pure CALE, and ii) comparing capsules from the top and bottom of stems.

Comparisons of hybrids and pure CALE: Limited data were obtained from pure individuals of either species during this project, so we supplemented these with data collected in other projects. We did not find any data collected on a per-capsule basis for CAHI, but Del Brummet, an undergraduate student at the University of Washington, examined seed production on a per-capsule basis in pure CALE. These capsules were obtained in 2012 from CALE plants grown experimentally without a host or with *Festuca roemeri* or *Eriophyllum lanatum* as a host (Delvin 2013). Although these samples were obtained in a different year and for a different purpose, the data are the most comparable to this trial as the experiment was conducted very close to the Prairie Habitat Restoration project at Glacial Heritage and on the same soil type. Two capsules

were collected from each of 36 plants, for a total of 72 capsules. Germination and viability testing were not conducted on these samples.

The following variables were compared between these datasets:

- Capsule mass (g)
- Seed mass (g)
- Seed mass (percentage of total mass)
- Seeds quantity (seeds per capsule)
- Seed density (seeds per gram)

We used a mixed effects model with maternal plant specified as a random effect and taxon (CALE x CAHI vs. CALE) specified as a fixed effect. We partitioned the variance to determine the proportion of variance explained by maternal plant identity.

Differences due to capsule location: These analyses focused on the capsules from putative CALE x CAHI hybrids sampled during this study. The following variables were examined:

- Capsule mass (g)
- Seed mass (g)
- Seed mass (percentage of total mass)
- Seeds quantity (seeds per capsule)
- Seed density (seeds per gram)
- Percentage live seed (germinated or were viable)
- Number of days until germination began
- Number of days until germination peaked
- Number of days until last germination occurred
- Length of germination period (days)

We used a mixed effects model with maternal plant specified as a random effect and capsule location (top vs. bottom of stem) specified as a fixed effect. We partitioned the variance to determine the proportion of variance explained by maternal plant identity.

Results

Hybrids vs. Pure CALE

Putative CALE x CAHI hybrids differed from CALE in all measured variables (Table 1). Capsules on hybrid plants were smaller and contained less than half as many seeds per capsule (Figure 2), though the seeds themselves were larger than in CALE.

The majority of the CALE x CAHI capsules contained properly formed seeds, but there were some instances of seed malformation (Figure 3). In some capsules, seeds appeared darkly discolored, perhaps due to mold (Figure 4). Many of these capsules also had low germination yields.

Maternal plant identity accounted for a sizable percentage of the variation in seed mass and seed quantity (seeds per capsule) (Table 1).

Table 1. Summary statistics comparing capsules from putative CALE x CAHI hybrids and pure CALE plants. The columns for 'CALE x CAHI' and 'CALE' report the mean value for each variable, '*P*' reports the statistical significance of the effect of taxon (values < 0.05 in bold), and 'Variation Among Plants' reports the percentage of the variance that is due to difference among maternal plants.

Variable	CALE x CAHI	CALE	*P*	Variation Among Plants
Total mass (g)	0.0335	0.0422	**<0.001**	11.9
Seed mass (g)	0.0095	0.0164	**<0.001**	34.5
Seed mass (% of total mass)	28.2	38.2	**<0.001**	21.0
Seed quantity (seeds per capsule)	82	187	**<0.001**	41.5
Seed density (seeds per gram)	8,837	12,352	**<0.001**	18.9

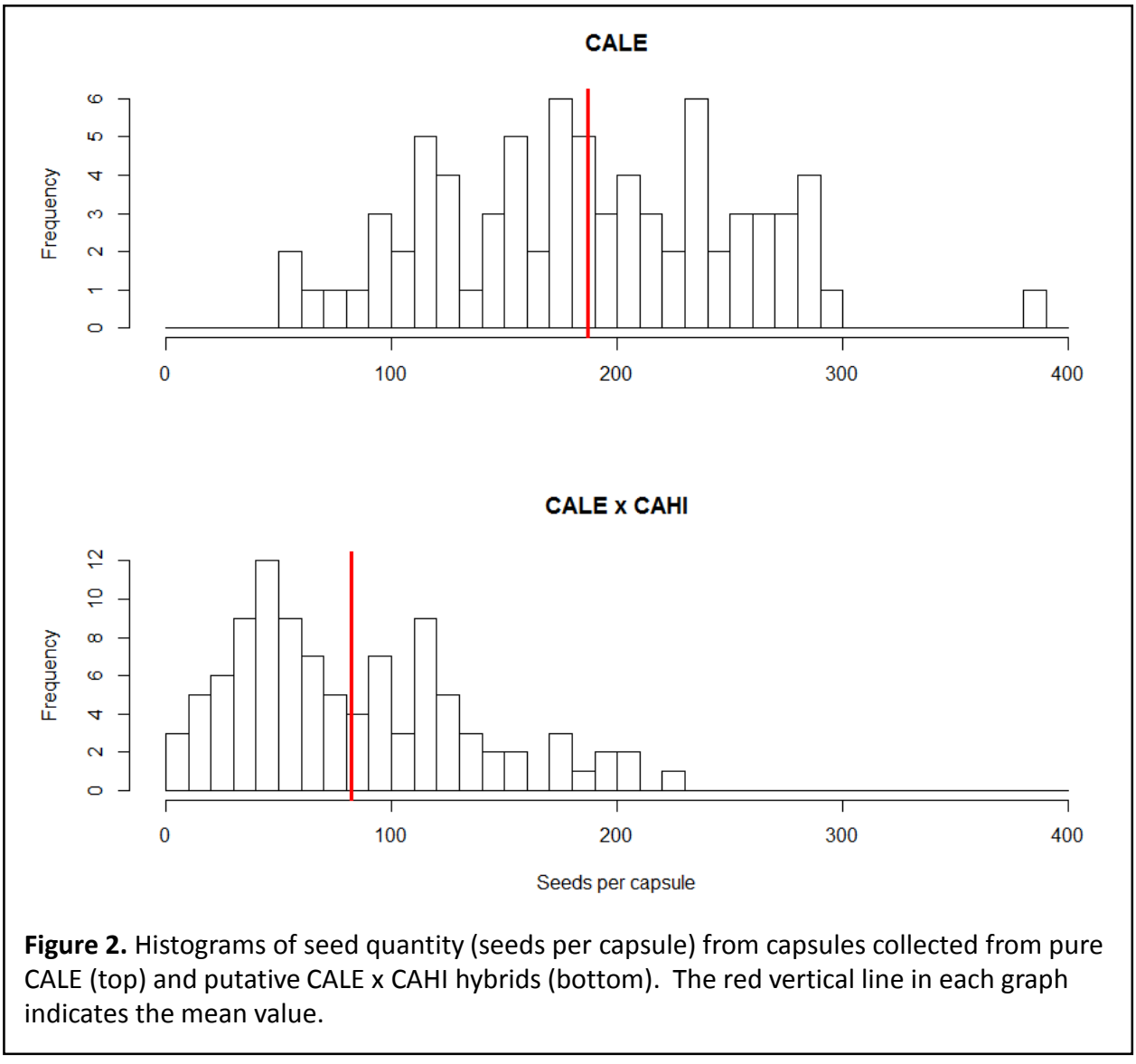

Figure 2. Histograms of seed quantity (seeds per capsule) from capsules collected from pure CALE (top) and putative CALE x CAHI hybrids (bottom). The red vertical line in each graph indicates the mean value.

Figure 3. A mixture of malformed and properly formed seeds from a single CALE x CAHI capsule (replicate A, bottom capsule, maternal plant 17).

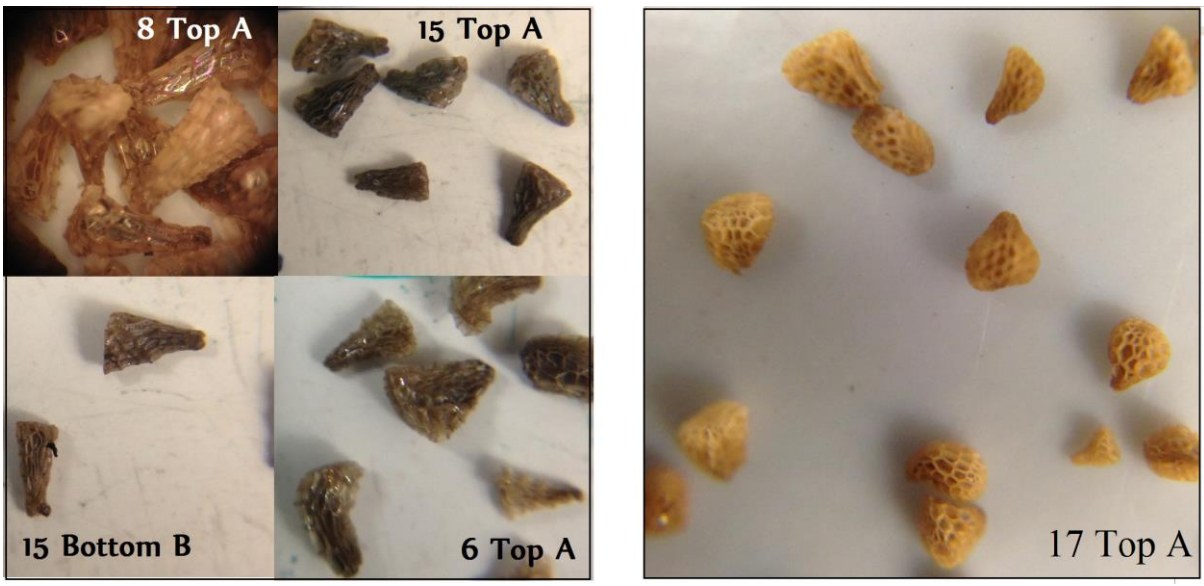

Figure 4. Discolored seeds with low germination yields (left) compared to healthy seeds (right). All images are from putative CALE x CAHI hybrids; the codes refer to the maternal plant ID, capsule location, and replicate.

Capsule Location

Effects of capsule location (Table 2) were much smaller than those among taxa. Capsules from the bottom of the stem tended to have fewer but heavier seeds than capsules from the top of the stem, and therefore had a significantly lower average seed density (Figure 5).

About three-quarters of all seeds were viable; this proportion did not differ with capsule location.

Germination began after an average of 36 days of stratification and peaked 14 days later. The average germination period was about 50 days long but was longer for capsules from the top of the stem, perhaps because they contained more seeds. For comparison, seeds from the four CALE capsules sampled in this study began to germinate after an average of 35 days, peaked 18 days later, and had a germination period of 64 days. Seeds from the four CAHI capsules sampled in this study began to germinate after 55 days, peaked 29 days later, and had a germination period of 61 days.

For variables such as total capsule mass, little of the variation occurred among maternal plants. In contrast, almost two-thirds of the variation in seed quantity (seeds per capsule) and more than half of the variation in when germination began were explained by the maternal plant from which the capsule was collected. For example, mean seed quantity ranged from 27 (plant 19) to 177 seeds per capsule (plant 12) (Figure 6). Percent germination ranged from 26% (plant 6) to 95% (plant 13). The number of days of stratification before germination began ranged from 25 (plant 16) to 53 (plant 10).

Table 2. Summary statistics comparing capsules from the top and bottom of flowering stems of putative CALE x CAHI hybrids. The columns for 'Top' and 'Bottom' report the mean value for each variable, '*P*' reports the statistical significance of the effect of capsule location (values < 0.05 in bold), and 'Variation Among Plants' reports the percentage of the variance that is due to difference among maternal plants.

Variable	Top	Bottom	*P*	Variation Among Plants
Total mass (g)	0.0315	0.0355	0.110	11.2
Seed mass (g)	0.0095	0.0095	0.947	42.6
Seed mass (% of total mass)	30.1	26.4	**0.018**	34.6
Seed quantity (seeds per capsule)	88	76	0.079	62.1
Seed density (seeds per gram)	9,825	7,849	**0.004**	31.9
Live seeds (% of total)	76.5	77.4	0.767	40.7
Days until germination began	36	37	0.567	54.2
Days until germination peaked	52	51	0.483	45.9
Days until germination ended	91	83	**0.041**	17.9
Length of germination period	55	47	**0.037**	13.1

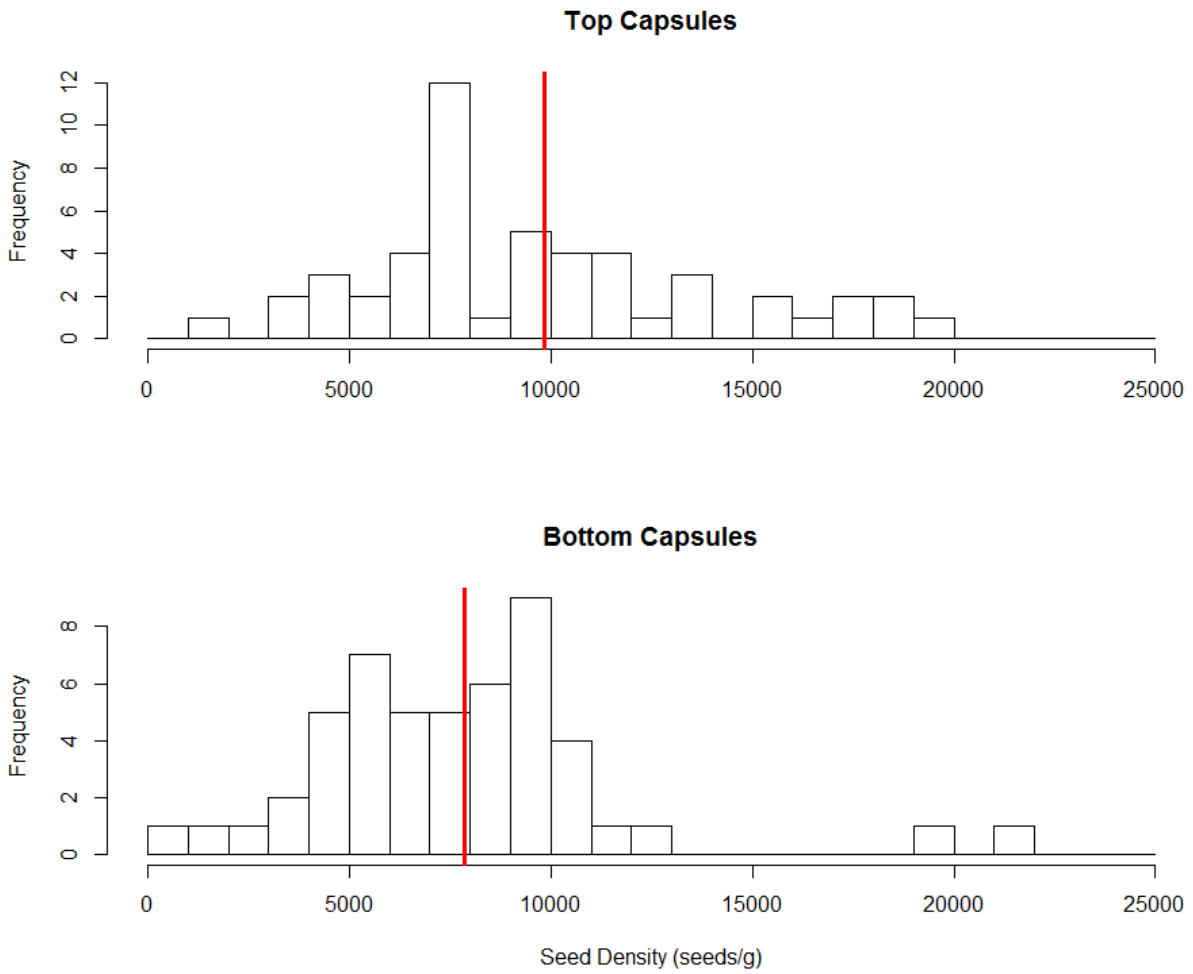

Figure 5. Histograms of seed density from capsules collected from the top and bottom of the stem of putative CALE x CAHI hybrids. The red vertical line in each graph indicates the mean value.

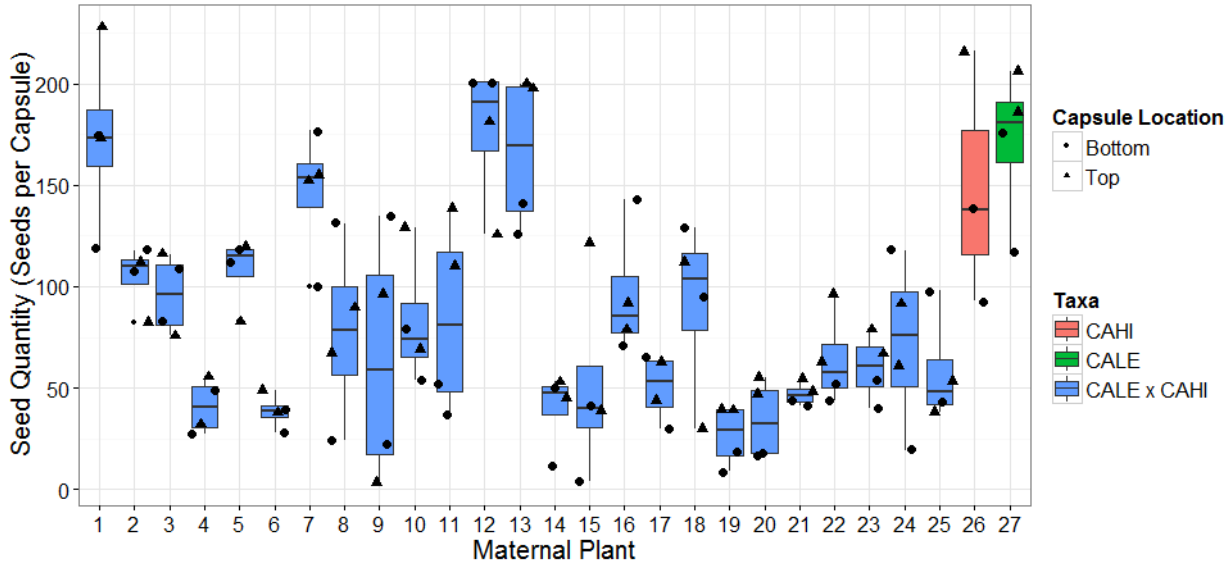

Figure 6. Seed quantity (seeds per capsule), by maternal plant. Note the CAHI and CALE (plants 26 and 27). Data are shown for each capsule; values are jittered horizontally around the center of each bar. One CAHI capsule with no seeds was omitted.

Discussion and Recommendations

Our results demonstrate that plants exhibiting CALE x CAHI morphological traits are able to produce viable seeds, though they produce many fewer seeds than CALE produces on average. One key limitation with this analysis is that we do not know the hybridization history of the sampled maternal plants. The plants were presumed to be F1 hybrids at the time of collection, but enough time elapsed between when the CALE and CAHI were seeded and when the fruiting stems were harvested that it is possible that they are F2 or later generations.

Maternal plant identity accounted for a sizable proportion of the variation in seed quantity (seeds per capsule) and when germination began. Differences among maternal plants may relate to genetic history (degree of hybridization, which species was the maternal parent of the plants sampled here and which was the pollen donor), but could also relate to spatial context, such as the pollinator community and 'hybridization pressure' (proximity of the two species).

It is intriguing to note how similar the germination patterns were for the putative hybrids and for CALE in terms of when germination began (after 35-37 days of stratification began) and peaked (14-18 days after germination began). In comparison to these, CAHI seeds did not begin to germinate until after 55 days of stratification and peak germination occurred 29 days after that. However, this is admittedly based on small datasets for CALE and CAHI.

Estimating the effect of a hybrid plant on the genetic structure of the next generation requires also knowing its total seed production, which is strongly influenced by how many capsules are produced (images of these maternal plants in Appendix 1 suggest this is comparable to capsule production by CALE) and knowing the proportion of the total seed production that the hybrid

represents. In a large CALE population, the few seeds produced by a hybrid could easily be swamped by those from pure CALE.

We were not able to rigorously compare the putative CALE x CAHI hybrids with pure CALE and CAHI. The CALE that we compared them against were grown in a different year and under different conditions, though at the same site. We do not know how much of the observed differences between them are due to these differences or to the genetic differences between pure plants of a species and hybrids. We have even less information about how these hybrids compare to pure CAHI as we do not have any information beyond that collected here about seed production and germination on a per-capsule basis in this species.

There are many facets of hybridization that require research. Clark (2015) suggested that CALE is more likely than CAHI to be a pollen donor, but this should be confirmed through controlled backcrosses. Spatial proximity of the two species is likely a key factor, as is the degree of phenological overlap in flowering between the species. Pollinator behavior is another area to be explored, not only in terms of pollinator preferences for pure and hybrid *Castilleja*, but also in terms of which historical pollinators are currently present in wild versus reintroduced *Castilleja* populations.

Upcoming Activities

Samples of surviving seedlings are currently growing in the Center for Urban Horticulture greenhouse. Leaf samples from 16 of the maternal lines and from more than a thousand germinants were sent in May 2015 to Drs. Jeremie Fant and Andrea Kramer at the Chicago Botanic Garden for a paternity analysis and/or other genetic analyses to confirm that plants showing intermediate CALE and CAHI traits are indeed hybrids and to identify which species was the pollen donor. Results of these analyses may provide further insight into the patterns reported here. For example, we would like to explore whether seed production and viability differ between hybrids where CALE is the maternal plant (CAHI the pollen donor) and hybrids where CAHI is the maternal plant (CALE the pollen donor).

As noted in the Introduction, a related project has been initiated by Dr. Tom Kaye (Institute for Applied Ecology) using controlled crosses among CALE and CAHI (Kaye 2014).

Acknowledgments

We thank Dapinder Jourha and Liesl Strand for assistance monitoring germination, Natalie Schmidt and Lauren Clark for on-site mentorship, Delaney Brummet for sharing his data, and Sierra Smith and Carl Elliot for providing guidance in determining appropriate germination protocols. Photos were provided by Peter Dunwiddie, Liesl Strand, and Loretta Fisher. Partial funding for this trial was provided by the US Fish and Wildlife Service through the Center for Natural Lands Management.

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Appendices

Appendix 1: Photographs of maternal plants and their seeds.

Photographs of maternal plants were taken by Peter Dunwiddie in Summer 2014 while collecting fruiting stems. Plants 1 through 25 are putative CALE x CAHI hybrids.

Photographs of seeds were taken by Loretta Fisher and/or Liesl Strand during seed testing in Winter 2015. Each image is of a subset of the seeds from one capsule, identified by the plant number, capsule location (top or bottom of stem) and replicate (A or B). Not all capsules were photographed.

Plant 1

Plant 2

Plant 3

Plant 4

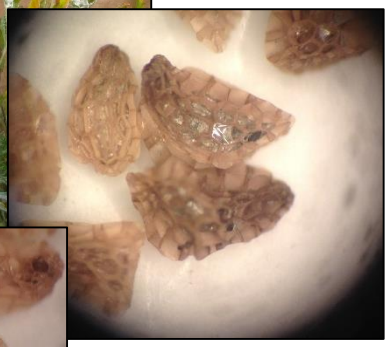
Plant 5

Plant 6

Plant 7

Plant 8

8 Top B



8 Bottom B

Plant 9

9 Top B

9 Bottom A

Plant 10

10 Top A

10 Bottom B

Plant 11

Plant 12

12 Top A

12 Bottom A

Plant 13

Plant 14

14 Top A

14 Bottom A

Plant 15

15 Top A

15 Bottom A

Plant 16

16 Top A

16 Bottom A

Plant 17

17 Top A

17 Bottom A

Plant 18

Plant 19

19 Top B

19 Bottom B

Plant 20

20 Top A

20 Bottom A

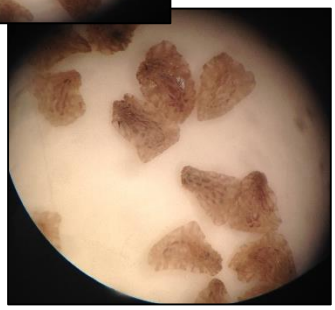
Plant 21

21 Top A

21 Bottom A

Plant 22

22 Top B



22 Bottom B

Plant 23

Plant 24

24 Top B

24 Bottom B

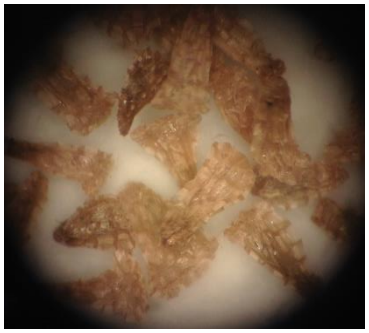
Plant 25

25 Top B

25 Bottom B

CAHI

CAHI Top B



CAHI Bottom B

CALE

CALE Top A

CALE Bottom B

Appendix 2: Seed data by capsule, for putative CALE x CAHI hybrids and pure CALE and CAHI.

This table reports the raw data for each capsule. Metadata are provided in a separate table below the data.

Taxa	Maternal Plant	Capsule Location	Replicate	Seed Quantity	Total Mass	Seed Mass	Seed Density	Proportion Seed Mass	Notes
CALE x CAHI	1	Top	A	173	0.042	0.019	9105	0.452	
CALE x CAHI	1	Top	B	228	0.028	0.013	17538	0.464	
CALE x CAHI	1	Bottom	A	174	0.04	0.014	12429	0.350	
CALE x CAHI	1	Bottom	B	119	0.037	0.013	9154	0.351	
CALE x CAHI	2	Top	A	82	0.037	0.011	7455	0.297	
CALE x CAHI	2	Top	B	112	0.037	0.014	8000	0.378	
CALE x CAHI	2	Bottom	A	118	0.047	0.017	6941	0.362	
CALE x CAHI	2	Bottom	B	108	0.044	0.015	7200	0.341	
CALE x CAHI	3	Top	A	116	0.025	0.01	11600	0.400	
CALE x CAHI	3	Top	B	76	0.023	0.004	19000	0.174	
CALE x CAHI	3	Bottom	A	83	0.031	0.009	9222	0.290	
CALE x CAHI	3	Bottom	B	109	0.036	0.011	9909	0.306	
CALE x CAHI	4	Top	A	55	0.024	0.005	11000	0.208	
CALE x CAHI	4	Top	B	32	0.021	0.005	6400	0.238	
CALE x CAHI	4	Bottom	A	27	0.028	0.005	5400	0.179	
CALE x CAHI	4	Bottom	B	49	0.031	0.009	5444	0.290	
CALE x CAHI	5	Top	A	120	0.048	0.018	6667	0.375	
CALE x CAHI	5	Top	B	83	0.019	0.007	11857	0.368	
CALE x CAHI	5	Bottom	A	118	0.037	0.012	9833	0.324	
CALE x CAHI	5	Bottom	B	112	0.034	0.01	11200	0.294	
CALE x CAHI	6	Top	A	38	0.021	0.002	19000	0.095	
CALE x CAHI	6	Top	B	49	0.02	0.003	16333	0.150	
CALE x CAHI	6	Bottom	A	39	0.022	0.002	19500	0.091	
CALE x CAHI	6	Bottom	B	28	0.019	0.004	7000	0.211	
CALE x CAHI	7	Top	A	152	0.038	0.011	13818	0.289	
CALE x CAHI	7	Top	B	155	0.048	0.021	7381	0.438	
CALE x CAHI	7	Bottom	A	100	0.042	0.015	6667	0.357	

Taxa	Maternal Plant	Capsule Location	Replicate	Seed Quantity	Total Mass	Seed Mass	Seed Density	Proportion Seed Mass	Notes
CALE x CAHI	7	Bottom	B	177	0.051	0.02	8850	0.392	
CALE x CAHI	8	Top	A	90	0.024	0.008	11250	0.333	
CALE x CAHI	8	Top	B	67	0.037	0.009	7444	0.243	
CALE x CAHI	8	Bottom	A	131	0.037	0.013	10077	0.351	
CALE x CAHI	8	Bottom	B	24	0.047	0.006	4000	0.128	
CALE x CAHI	9	Top	A	3	0.009	0.002	1500	0.222	
CALE x CAHI	9	Top	B	96	0.027	0.013	7385	0.481	
CALE x CAHI	9	Bottom	A	22	0.023	0.008	2750	0.348	
CALE x CAHI	9	Bottom	B	135	0.062	0.015	9000	0.242	
CALE x CAHI	10	Top	A	129	0.047	0.018	7167	0.383	
CALE x CAHI	10	Top	B	69	0.029	0.009	7667	0.310	
CALE x CAHI	10	Bottom	A	79	0.042	0.008	9875	0.190	
CALE x CAHI	10	Bottom	B	54	0.04	0.009	6000	0.225	
CALE x CAHI	11	Top	A	139	0.032	0.012	11583	0.375	
CALE x CAHI	11	Top	B	110	0.031	0.01	11000	0.323	
CALE x CAHI	11	Bottom	A	37	0.017	0.004	9250	0.235	
CALE x CAHI	11	Bottom	B	52	0.077	0.007	7429	0.091	
CALE x CAHI	12	Top	A	126	0.021	0.007	18000	0.333	
CALE x CAHI	12	Top	B	181	0.033	0.012	15083	0.364	
CALE x CAHI	12	Bottom	A	201	0.058	0.022	9136	0.379	
CALE x CAHI	12	Bottom	B	201	0.05	0.021	9571	0.420	
CALE x CAHI	13	Top	A	198	0.046	0.021	9429	0.457	
CALE x CAHI	13	Top	B	200	0.047	0.02	10000	0.426	
CALE x CAHI	13	Bottom	A	141	0.039	0.013	10846	0.333	
CALE x CAHI	13	Bottom	B	126	0.04	0.012	10500	0.300	
CALE x CAHI	14	Top	A	45	0.026	0.006	7500	0.231	
CALE x CAHI	14	Top	B	53	0.024	0.007	7571	0.292	
CALE x CAHI	14	Bottom	A	50	0.038	0.007	7143	0.184	
CALE x CAHI	14	Bottom	B	11	0.016	0.002	5500	0.125	
CALE x CAHI	15	Top	A	121	0.088	0.011	11000	0.125	
CALE x CAHI	15	Top	B	39	0.016	0.005	7800	0.313	

Taxa	Maternal Plant	Capsule Location	Replicate	Seed Quantity	Total Mass	Seed Mass	Seed Density	Proportion Seed Mass	Notes
CALE x CAHI	15	Bottom	A	41	0.03	0.005	8200	0.167	
CALE x CAHI	15	Bottom	B	4	0.028	0.009	444	0.321	
CALE x CAHI	16	Top	A	79	0.014	0.004	19750	0.286	
CALE x CAHI	16	Top	B	92	0.019	0.006	15333	0.316	
CALE x CAHI	16	Bottom	A	143	0.029	0.013	11000	0.448	
CALE x CAHI	16	Bottom	B	71	0.02	0.008	8875	0.400	
CALE x CAHI	17	Top	A	63	0.046	0.012	5250	0.261	
CALE x CAHI	17	Top	B	44	0.041	0.009	4889	0.220	
CALE x CAHI	17	Bottom	A	65	0.054	0.011	5909	0.204	
CALE x CAHI	17	Bottom	B	30	0.028	0.006	5000	0.214	
CALE x CAHI	18	Top	A	30	0.022	0.008	3750	0.364	
CALE x CAHI	18	Top	B	112	0.055	0.012	9333	0.218	
CALE x CAHI	18	Bottom	A	95	0.053	0.016	5938	0.302	
CALE x CAHI	18	Bottom	B	129	0.038	0.006	21500	0.158	
CALE x CAHI	19	Top	A	39	0.034	0.007	5571	0.206	
CALE x CAHI	19	Top	B	39	0.037	0.01	3900	0.270	
CALE x CAHI	19	Bottom	A	9	0.025	0.002	4500	0.080	
CALE x CAHI	19	Bottom	B	19	0.027	0.01	1900	0.370	
CALE x CAHI	20	Top	A	55	0.024	0.004	13750	0.167	
CALE x CAHI	20	Top	B	47	0.025	0.005	9400	0.200	
CALE x CAHI	20	Bottom	A	18	0.015	0.003	6000	0.200	Germination data for these two capsules averaged due to accidental swapping of data sheets during germination testing. (Mislabeled)
CALE x CAHI	20	Bottom	B	16	0.023	0.004	4000	0.174	Germination data for these two capsules averaged due to accidental swapping of data sheets during germination testing.

Taxa	Maternal Plant	Capsule Location	Replicate	Seed Quantity	Total Mass	Seed Mass	Seed Density	Proportion Seed Mass	Notes
									(Mislabeled)
CALE x CAHI	21	Top	A	48	0.018	0.006	8000	0.333	
CALE x CAHI	21	Top	B	54	0.023	0.006	9000	0.261	
CALE x CAHI	21	Bottom	A	44	0.029	0.005	8800	0.172	
CALE x CAHI	21	Bottom	B	41	0.033	0.006	6833	0.182	
CALE x CAHI	22	Top	A	63	0.018	0.005	12600	0.278	
CALE x CAHI	22	Top	B	96	0.031	0.007	13714	0.226	
CALE x CAHI	22	Bottom	A	44	0.026	0.01	4400	0.385	
CALE x CAHI	22	Bottom	B	52	0.027	0.008	6500	0.296	
CALE x CAHI	23	Top	A	67	0.049	0.015	4467	0.306	
CALE x CAHI	23	Top	B	79	0.028	0.01	7900	0.357	
CALE x CAHI	23	Bottom	A	40	0.025	0.005	8000	0.200	
CALE x CAHI	23	Bottom	B	54	0.036	0.007	7714	0.194	
CALE x CAHI	24	Top	A	91	0.039	0.014	6500	0.359	
CALE x CAHI	24	Top	B	61	0.03	0.013	4692	0.433	
CALE x CAHI	24	Bottom	A	118	0.048	0.0125	9440	0.260	
CALE x CAHI	24	Bottom	B	19	0.021	0.004	4750	0.190	
CALE x CAHI	25	Top	A	38	0.022	0.006	6333	0.273	
CALE x CAHI	25	Top	B	53	0.031	0.005	10600	0.161	
CALE x CAHI	25	Bottom	A	43	0.033	0.009	4778	0.273	
CALE x CAHI	25	Bottom	B	98	0.04	0.012	8167	0.300	
CAHI	26	Top	A	0	0.008	0			Capsule empty
CAHI	26	Top	B	216	0.028	0.013	16615	0.464	
CAHI	26	Bottom	A	93	0.024	0.006	15500	0.250	
CAHI	26	Bottom	B	138	0.027	0.012	11500	0.444	
CALE	27	Top	A	206	0.036	0.019	10842	0.528	
CALE	27	Top	B	186	0.028	0.003	62000	0.107	
CALE	27	Bottom	A	117	0.013	0.005	23400	0.385	
CALE	27	Bottom	B	176	0.068	0.018	9778	0.265	

Metadata for capsule data:

Column	Description	Levels or Range	Data Type	Units
Taxa	Taxon sampled	CALE, CAHI, CALE x CAHI	Nominal	
Maternal Plant	Unique ID of maternal plant	1 through 27	Nominal	
Capsule Location	Where on fruiting stem capsule was taken from	Top, Bottom	Nominal	
Replicate	Which capsule on that plant at that location	A, B	Nominal	
Seed Quantity	Number of seeds in capsule	0-228	Integer	Seeds / capsule
Total Mass	Mass of capsule, including seeds	0.008-0.088		Grams
Seed Mass	Mass of seeds	0-0.022		Grams
Seed Density	Seed Quantity divided by Seed Mass	444-62,000		Seeds / gram
Proportion Seed Mass	Seed Mass divided by Total Mass	0.080-0.528		
Notes	Written notes about sample		Text	

Appendix 3: Germination data by capsule, for putative CALE x CAHI hybrids and pure CALE and CAHI.

This table reports the germination data for each capsule. Metadata are provided in a separate table below the data.

Taxa	Maternal Plant	Capsule Location	Replicate	Start	Mode	End	Germination Length	Germinants	Viable Seeds	Percent Germination	Percent Live
CALE x CAHI	1	Top	A	40	56	111	71	138	0	79.77	79.77
CALE x CAHI	1	Top	B	40	56	90	50	123	0	53.95	53.95
CALE x CAHI	1	Bottom	A	41	57	112	71	160	0	91.95	91.95
CALE x CAHI	1	Bottom	B	50	50	91	41	102	0	85.71	85.71
CALE x CAHI	2	Top	A	38	54	109	71	79	0	96.34	96.34
CALE x CAHI	2	Top	B	24	47	116	92	106	0	94.64	94.64
CALE x CAHI	2	Bottom	A	24	38	74	50	110	0	93.22	93.22
CALE x CAHI	2	Bottom	B	24	47	74	50	87	0	80.56	80.56
CALE x CAHI	3	Top	A	49	70	123	74	116	0	100.00	100.00
CALE x CAHI	3	Top	B	48	59	98	50	50	0	65.79	65.79
CALE x CAHI	3	Bottom	A	48	72	98	50	75	0	90.36	90.36
CALE x CAHI	3	Bottom	B	49	68	102	53	92	0	84.40	84.40
CALE x CAHI	4	Top	A	27	40	76	49	31	0	56.36	56.36
CALE x CAHI	4	Top	B	27	49	90	63	25	0	78.13	78.13
CALE x CAHI	4	Bottom	A	40	40	111	71	24	0	88.89	88.89
CALE x CAHI	4	Bottom	B	27	40	90	63	40	0	81.63	81.63
CALE x CAHI	5	Top	A	38	47	77	39	85	0	70.83	70.83
CALE x CAHI	5	Top	B	38	61	68	30	43	1	51.81	53.01
CALE x CAHI	5	Bottom	A	38	48	92	54	90	0	76.27	76.27
CALE x CAHI	5	Bottom	B	38	47	70	32	101	0	90.18	90.18
CALE x CAHI	6	Top	A	47	69	69	22	10	0	26.32	26.32
CALE x CAHI	6	Top	B	47	69	109	62	10	0	20.41	20.41
CALE x CAHI	6	Bottom	A	38	88	116	78	11	0	28.21	28.21
CALE x CAHI	6	Bottom	B	38	47	69	31	9	0	32.14	32.14
CALE x CAHI	7	Top	A	40	47	97	57	146	0	96.05	96.05
CALE x CAHI	7	Top	B	40	49	90	50	145	0	93.55	93.55
CALE x CAHI	7	Bottom	A	40	56	111	71	89	0	89.00	89.00

Taxa	Maternal Plant	Capsule Location	Replicate	Start	Mode	End	Germination Length	Germinants	Viable Seeds	Percent Germination	Percent Live
CALE x CAHI	7	Bottom	B	40	49	118	78	135	0	76.27	76.27
CALE x CAHI	8	Top	A	48	71	89	41	49	0	54.44	54.44
CALE x CAHI	8	Top	B	31	40	61	30	43	0	64.18	64.18
CALE x CAHI	8	Bottom	A	58	71	89	31	78	0	59.54	59.54
CALE x CAHI	8	Bottom	B	31	40	80	49	21	0	87.50	87.50
CALE x CAHI	9	Top	A	40	40	59	19	3	0	100.00	100.00
CALE x CAHI	9	Top	B	33	60	91	58	73	0	76.04	76.04
CALE x CAHI	9	Bottom	A	40	50	82	42	20	0	90.91	90.91
CALE x CAHI	9	Bottom	B	33	42	104	71	109	0	80.74	80.74
CALE x CAHI	10	Top	A	50	63	82	32	122	0	94.57	94.57
CALE x CAHI	10	Top	B	50	63	82	32	64	0	92.75	92.75
CALE x CAHI	10	Bottom	A	50	60	73	23	40	0	50.63	50.63
CALE x CAHI	10	Bottom	B	63	63	82	19	49	0	90.74	90.74
CALE x CAHI	11	Top	A	41	48	98	57	114	0	82.01	82.01
CALE x CAHI	11	Top	B	41	48	98	57	75	0	68.18	68.18
CALE x CAHI	11	Bottom	A	31	50	59	28	26	0	70.27	70.27
CALE x CAHI	11	Bottom	B	38	38	59	21	45	0	86.54	86.54
CALE x CAHI	12	Top	A	28	59	151	123	114	0	90.48	90.48
CALE x CAHI	12	Top	B	28	59	82	54	161	0	88.95	88.95
CALE x CAHI	12	Bottom	A	28	50	82	54	186	0	92.54	92.54
CALE x CAHI	12	Bottom	B	40	50	70	30	172	0	85.57	85.57
CALE x CAHI	13	Top	A	21	48	92	71	194	0	97.98	97.98
CALE x CAHI	13	Top	B	38	61	97	59	185	0	92.50	92.50
CALE x CAHI	13	Bottom	A	38	48	79	41	135	0	95.74	95.74
CALE x CAHI	13	Bottom	B	38	61	92	54	120	0	95.24	95.24
CALE x CAHI	14	Top	A	25	38	56	31	23	0	51.11	51.11
CALE x CAHI	14	Top	B	25	38	56	31	43	0	81.13	81.13
CALE x CAHI	14	Bottom	A	25	38	88	63	35	0	70.00	70.00
CALE x CAHI	14	Bottom	B	38	38	54	16	7	0	63.64	63.64
CALE x CAHI	15	Top	A	25	38	74	49	67	0	55.37	55.37
CALE x CAHI	15	Top	B	25	56	88	63	25	0	64.10	64.10

Taxa	Maternal Plant	Capsule Location	Replicate	Start	Mode	End	Germination Length	Germinants	Viable Seeds	Percent Germination	Percent Live
CALE x CAHI	15	Bottom	A	25	47	74	49	28	0	68.29	68.29
CALE x CAHI	15	Bottom	B	47	47	56	9	2	0	50.00	50.00
CALE x CAHI	16	Top	A	25	38	69	44	64	0	81.01	81.01
CALE x CAHI	16	Top	B	25	38	88	63	77	0	83.70	83.70
CALE x CAHI	16	Bottom	A	25	47	88	63	108	0	75.52	75.52
CALE x CAHI	16	Bottom	B	25	47	69	44	70	0	98.59	98.59
CALE x CAHI	17	Top	A	40	50	70	30	52	0	82.54	82.54
CALE x CAHI	17	Top	B	28	59	89	61	31	0	70.45	70.45
CALE x CAHI	17	Bottom	A	28	28	59	31	48	0	73.85	73.85
CALE x CAHI	17	Bottom	B	33	42	73	40	17	0	56.67	56.67
CALE x CAHI	18	Top	A	38	48	111	73	27	0	90.00	90.00
CALE x CAHI	18	Top	B	28	51	139	111	91	0	81.25	81.25
CALE x CAHI	18	Bottom	A	30	51	132	102	95	0	100.00	100.00
CALE x CAHI	18	Bottom	B	38	48	79	41	17	0	13.18	13.18
CALE x CAHI	19	Top	A	44	44	76	32	20	0	51.28	51.28
CALE x CAHI	19	Top	B	38	56	95	57	20	0	51.28	51.28
CALE x CAHI	19	Bottom	A	38	38	88	50	9	0	100.00	100.00
CALE x CAHI	19	Bottom	B	38	56	56	18	12	0	63.16	63.16
CALE x CAHI	20	Top	A	40	63	82	42	49	0	89.09	89.09
CALE x CAHI	20	Top	B	40	63	82	42	47	0	100.00	100.00
CALE x CAHI	20	Bottom	A	28	73	82	54	13	0	72.22	72.22
CALE x CAHI	20	Bottom	B	28	73	82	54	13	0	81.25	81.25
CALE x CAHI	21	Top	A	38	56	109	71	47	0	97.92	97.92
CALE x CAHI	21	Top	B	35	35	88	53	46	0	85.19	85.19
CALE x CAHI	21	Bottom	A	38	47	88	50	35	0	79.55	79.55
CALE x CAHI	21	Bottom	B	38	54	109	71	37	0	90.24	90.24
CALE x CAHI	22	Top	A	28	50	73	45	40	0	63.49	63.49
CALE x CAHI	22	Top	B	28	42	82	54	68	0	70.83	70.83
CALE x CAHI	22	Bottom	A	28	50	73	45	34	0	77.27	77.27
CALE x CAHI	22	Bottom	B	28	42	73	45	44	0	84.62	84.62
CALE x CAHI	23	Top	A	48	48	77	29	63	0	94.03	94.03

Taxa	Maternal Plant	Capsule Location	Replicate	Start	Mode	End	Germination Length	Germinants	Viable Seeds	Percent Germination	Percent Live
CALE x CAHI	23	Top	B	48	48	132	84	65	0	82.28	82.28
CALE x CAHI	23	Bottom	A	38	38	77	39	31	0	77.50	77.50
CALE x CAHI	23	Bottom	B	38	61	77	39	50	0	92.59	92.59
CALE x CAHI	24	Top	A	38	68	149	111	66	0	72.53	72.53
CALE x CAHI	24	Top	B	38	61	89	51	49	0	80.33	80.33
CALE x CAHI	24	Bottom	A	48	61	87	39	106	0	89.83	89.83
CALE x CAHI	24	Bottom	B	48	61	68	20	10	0	52.63	52.63
CALE x CAHI	25	Top	A	31	48	95	64	33	0	86.84	86.84
CALE x CAHI	25	Top	B	26	40	71	45	39	0	73.58	73.58
CALE x CAHI	25	Bottom	A	31	48	71	40	39	0	90.70	90.70
CALE x CAHI	25	Bottom	B	26	48	89	63	72	0	73.47	73.47
CAHI	26	Top	A								
CAHI	26	Top	B	54	87	137	83	129	0	59.72	59.72
CAHI	26	Bottom	A	54	87	105	51	45	0	48.39	48.39
CAHI	26	Bottom	B	56	77	105	49	98	0	71.01	71.01
CALE	27	Top	A	28	54	86	58	186	0	90.29	90.29
CALE	27	Top	B	40	61	89	49	176	0	94.62	94.62
CALE	27	Bottom	A	28	37	139	111	101	0	86.32	86.32
CALE	27	Bottom	B	45	58	84	39	165	0	93.75	93.75

Metadata for germination data:

Column	Description	Levels or Range	Data Type	Units
Taxa	Taxon sampled	CALE, CAHI, CALE x CAHI	Nominal	
Maternal Plant	Unique ID of maternal plant	1 through 27	Nominal	
Capsule Location	Where on fruiting stem capsule was taken from	Top, Bottom	Nominal	
Replicate	Which capsule on that plant at that location	A, B	Nominal	
Start	Days of stratification until germination began	21-63	Integer	Days
Mode	Days of stratification until germination peaked	28-88	Integer	Days
End	Days of stratification until germinated ended	54-151	Integer	Days
Germination Length	Total length of germination period (End minus Start)	9-123	Integer	Days
Germinants	Number of germinants tallied	2-194	Integer	#
Viable Seeds	Number of non-germinating seeds found to be viable through tetrazolium staining	0-1	Integer	#
Percent Germination	Germinants divided by Seed Quantity	13.18-100.00		%
Percent Live	(Germinants + Viable Seeds) divided by Seed Quantity	13.18-100.00		%