Germination biology of *Viola conspersa*, a native woodland violet

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Introduction:

Figure 1: *Viola conspersa*, the study species*

Viola conspersa, the American dog violet, is a native woodland violet. Its range extends throughout much of Canada and the eastern U.S.[1]. Though it has a large native range, *V. conspersa* is listed as threatened in Illinois[2].

V. conspersa is a perennial that employs a mixed reproductive strategy (i.e., it produces two types of flowers, chasmogamous (CH) and cleistogamous (CL) flowers). CH flowers are showy and reproduce primarily by cross-pollination, while CL flowers remain closed and reproduce solely by self-pollination. The presence of both types of flowers is thought to maximize reproductive output[3].

At this point, though several studies have examined differences in morphology and germination success between CH and CL seeds in several *Viola* and *Impatiens* species[3,4],no such study has been conducted for *V. conspersa*. As this species is threatened in Illinois, it is important to conduct such a study to better understand the reproductive dynamics of the plant and to help inform management decisions.

Additionally, it is incredibly important to ascertain the conditions that facilitate germination in *V. conspersa*. For instance, studies have shown that physical dormancy in certain herbaceous species can be broken by a period of cold stratification to mimic overwintering conditions[5]. Identifying *V. conspersa* as a species requiring cold stratification could help facilitate ex-situ conservation methods (i.e., cultivation of the species in a laboratory or greenhouse setting).

Objectives:

Experiment #1:

•To compare morphology (i.e., mass) of different seed types (CH vs. CL)

•To determine whether morphological differences between seed types contribute to differential germination success and seedling recruitment

Experiment #2

•To identify optimal environmental conditions for germination

Methods:

Experiment #1:

•Fruits were collected from McDonald Woods, and seeds were obtained (along with seeds collected by Dr. Vitt in 2008)

•Seeds were massed using a Mettler Toledo balance

•100 CH seeds were planted inside a seed basket in a McDonald Woods Site

•Seed basket was monitored weekly for seedling growth

Experiment #2:

Trial	Conditions Tested
A	Length of Cold Strat (2 vs. 4 vs. 6 weeks) Light vs. Dark CH vs. CL seeds
B	Temperature (20.6°C vs. 4°C) CH vs. CL seeds Soil vs. Filter Paper (as growth medium)

Figure 2: 4°C treatment groups from Trial A and Trial B

•For each unique combination of conditions (e.g., Trial A/4 weeks/Dark/CH Seeds), 3 petri dishes were prepared with 10 seeds each (600 seeds total)

•For Trial A, seeds were placed in a refrigerator set at 4°C for the duration of the cold treatment

• At the end of the cold stratification period, seeds were removed and placed in a 20.6°C incubator with 12 hour day and night periods

•Seeds in light treatments were monitored weekly for signs of germination (i.e., split seed coat, enlarged endosperm, emerging radicle)

•At the end of the study (5 weeks), seeds in dark treatments were removed from their foil coverings and monitored for germination

Abstract:

This study examined the morphological differences between seeds produced by chasmogamous and cleistogamous fruits of *Viola conspersa* and sought to compare the relative reproductive success of each flower type based on seed germination and seedling recruitment rates in the field. No statistically significant difference was found between masses of the two seed types, though the average CH seed mass was higher than the average CL seed mass. Field plantings of CH seeds were unsuccessful, and no plantings were attempted for CL seeds. In addition, a cold stratification experiment was performed to determine the optimal conditions for germination for this species. Very few seeds germinated, and the only conditions that seemed to consistently facilitate germination were a four week cold stratification period and absence of light. A seed viability test using 2,3,5-triphenyl tetrazolium chloride confirmed that the majority of seeds used were not viable.

Results:

Experiment #1 :

- **Seed Mass Data**: Analysis of Variance (ANOVA) Single Factor

Average Mass of CH Seeds	0.000575 grams
CH Variance	7.01E-08
Average Mass of CL Seeds	0.000525 grams
CL Variance	4.67E-08
P-Value	0.086453

- **Field Planting Data**: No CH seedlings emerged from soil
  No CL seeds were planted

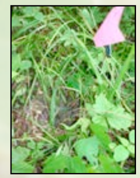

Figure 3: Seed basket with seeds planted in McDonald Woods

Experiment #2:

- **Germination Count** (after 5 weeks): 8 seeds (all from Trial A)
- Treatment groups with germinating seeds:
  1. 4 week cold stratification, CL seeds, light conditions → 2 germinating seeds
  2. 4 week cold stratification, CL seeds, dark conditions → 1 germinating seed
  3. 4 week cold stratification, CH seeds, dark conditions → 5 germinating seeds
- Note: 6 week treatments still have not been removed from the refrigerator or foil

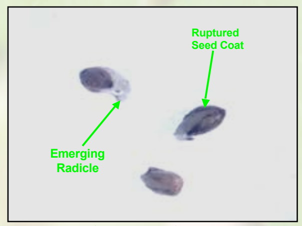

Figure 5: Germinating seeds from Trial A's 4-week dark treatment

Seed Viability Assessment:

A seed viability test using 2,3,5-triphenyl tetrazolium chloride (TTC) was performed on 60 seeds. Protocols were a variation of those outlined in *Techniques for Pollination Biologists*[6].

Figure 6: A- Administering TTC to bisected seeds
B- Positive Test (pink coloration)
C- Negative Test (absence of pink)

Procedure:
1. Tested 3 different seed ages
2. Soaked seeds overnight between moist sheets of filter paper
3. Bisected seed coats and embryos with a razor
4. Administered 400 μL of TTC to dishes housing 3 seeds each

TTC Test Results by Seed Age

Seed Age	% pink after 1 hour	% pink after 2 hours
2 weeks	75.0%	88.3%
1-2 months	50.0%	55.2%
1 year	6.9%	10.3%

TTC Test Results by Seed Age and Type

Seed Age/Type	% pink after 1 hour	% pink after 2 hours
1-2 months/CH	20.7%	24.1%
1-2 months/CL	79.3%	86.2%
1 year/CH	0.0%	0.0%
1 year/CL	13.8%	20.7%

Note: All 2 week seeds were CL

Discussion:

Experiment #1:

- Though the average CH seed mass was greater than the average CL seed mass, this difference was not statistically significant (P value > 0.05)
- CH seeds did not germinate on site, indicating…
  1. the seeds were too immature to germinate
     and/or
  2. the seeds were in a state of physical dormancy and required a period of cold stratification to germinate
     and/or
  3. some other field condition was inadequate to facilitate germination and seedling growth
- Thus, based on our results, we could not ascertain any significant morphological difference (based on mass) between CH and CL seeds or compare their reproductive success rates

Experiment #2:

- As only 8 out of 600 seeds germinated, it is evident that…
  1. some laboratory condition was suboptimal (e.g., fungal growth may have killed many of the seeds)
     and/or
  2. many of the seeds were immature or not viable
- However, among the 8 seeds that did germinate, a couple trends emerged…
- 8/8 germinating seeds were from the 4 week group, which may indicate that 4 weeks is an adequate or even optimal time period for cold stratification for this species
- 6/8 seeds germinated in the dark, possibly indicating that *V. conspersa* seeds require dark conditions (such as they would experience buried under the soil) to germinate

Seed Viability Assessment:

- The data indicates that *V. conspersa* seeds desiccate over time
- Thus, as 2/3 of the seeds used in Experiment #2 were 1-2 months old and 1/3 of the seeds were 1 year old, many of these seeds were not viable and could not germinate, even under optimal conditions
- Among the seeds tested, a higher percentage of CL seeds were viable than CH seeds, though a larger sample size is needed to confirm this result

Future Research:

Figure 7: Trial B seed with prominent elaiosome

Throughout the course of this study, prominent elaiosomes were observed on many seeds. Elaiosomes are lipid-rich structures that ants consume before dispersing seeds. A 1980 paper by Culver and Beattie suggests that elaiosome removal and seed coat scarification by ants may allow nutrients to better permeate the seed coat and facilitate germination in *Viola odorata and Viola hirta*[7]. As such, research involving artificial seed coat scarification and elaiosome removal from *V. conspersa* seeds should be considered.

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*Photo in Figure 1 courtesy of the Connecticut Botanical Society