

GERMINATION REQUIREMENTS OF THE EARLY-BLUE VIOLET (*VIOLA ADUNCA*)

Kama N. Almasi and Friedric P. Kollmann

Biology Department, University of Wisconsin – Stevens Point
800 Reserve St., Stevens Point, WI 54481
Kama.Almasi@uwsp.edu

ABSTRACT

Knowledge of seed germination requirements and other life history characteristics is important for successful management and restoration of native habitat. Little is known of the germination requirements of *Viola adunca* (Violaceae), whose population status is unknown in the mid-west, but declining in the west. We conducted a set of experiments examining the effect of soil type, soil pH, light and temperature on percent germination in *V. adunca*. We found that soil type and temperature regime had especially strong effects on percent germination, while light and pH also affected germination to a lesser degree. *Viola* spp. are declining in at least some habitats in Wisconsin, and *V. adunca* is being used as a restoration plant in the Pacific Northwest, so this research has potential for use in conservation and restoration activities in the Great Lakes region.

KEYWORDS: *Viola adunca*, Violaceae germination, restoration

INTRODUCTION

Biologists consider destruction of native habitat to be the worst threat to biodiversity and natural ecosystems. Along with other human-caused changes, it is believed to be causing a mass extinction of living species (Myers 1986). A recent United Nations report by 1,100 scientists estimates that in the next three decades the earth will lose over 70% of its natural habitats (UNEP 2002).

With such high rates of biodiversity and natural resource loss, conservation and restoration of natural habitats are growing in importance. For many habitats and species, restoration and reintroduction will be the only way in which they may survive. Unfortunately successful restoration projects have been rare (Lockwood and Pimm 1999) and, despite numerous attempts to restore habitat, the ecology of restoration is still not well understood. It is only in recent years that restoration practitioners have begun to use ecological principles to plan and implement their projects (Perrow and Davy 2002).

One application of ecological principles to restoration and conservation is the incorporation of basic life history research in management and restoration plans; for example, an understanding of germination and growth requirements is necessary for successful seeding and transplantation. Little is known about the specific germination requirements for many restoration species (though see Tieu et al. 1999 and Dixon et al. 1995, for studies on smoke enhancement of germination). We investigated the germination requirements for *Viola adunca* J.E. Smith (Violaceae), a widespread perennial plant found in meadows and prairies

throughout western and midwestern North America (Gleason and Cronquist 1991).

Relatively little is known of the status of *V. adunca* populations. It is listed as a species of “special concern” in Massachusetts (<http://www.mass.gov/dfwele/dfw/nhesp/nhrare.htm>) and in Washington state, the Burke Museum reports its conservation status as “not tracked” (<http://biology.burke.washington.edu/herbarium/imagecollection.php?Genus=ViolaandSpecies=adunca>). Populations have been declining in Oregon (Pickering et al. 2000) and Washington (Black and Vaughan 2005). This is disturbing, because *V. adunca* is a restoration plant in the Pacific Northwest as it is the sole host plant for the larval stage of the federally threatened Oregon silverspot butterfly, *Speyeria zerene* ssp. *hippolyta*.

Populations of *Viola* spp. have been declining in wet-mesic and northern upland forest habitats of Wisconsin during the past half century (Bushman 2006; Wiegmann and Waller 2006). Basic and applied ecological research on *Viola* spp. is needed if we are to better understand and conserve this important genus. Research on life history characteristics, including germination requirements of native plants, is essential for successful restoration. Thus an understanding of the germination requirements of *V. adunca* will be useful for managers and restoration ecologists of native prairies, bracken grasslands, and northern upland forests in which *V. adunca* is a major habitat component (WisFlora: <http://www.botany.wisc.edu/wisflora/>).

The conditions required to break seed dormancy can be complex. Factors affecting germination and dormancy may include a specific light:dark regime, temperature regime, and soil properties, among other things. The properties required to break dormancy may even differ between seeds produced in different years by the same species (Baskin and Baskin 1995). Relatively little is known about the germination requirements of *V. adunca*. A number of nurseries carry either the live plants or seed, but a request for germination information resulted in sparse information. Two nurseries suggested that *V. adunca* requires cold stratification. The Oregon Zoo has had some success with leaving seeds outside to overwinter in flats of soil (R. Hanes, pers. comm.). The only paper in the literature that discusses *V. adunca* germination reports zero success for the species under a series of treatments (Drake et al. 1998).

No information could be found detailing indoor propagation of *V. adunca*, as might be useful for restoration ecologists and researchers. Various techniques have been reported for other *Viola* species; for example, a period of dark has been found beneficial in increasing germination success of some *Viola* species (Doohan et al. 1991). Soaking seed with gibberellic acid can also aid in germination of some *Viola* species (H. Ballard, pers. comm.), but getting the concentration and timing right can be difficult (Riley 1987).

We tested germination of *V. adunca* seeds under a variety of soil, light and temperature regimes to see whether germination could be induced ex-situ in a greenhouse setting, in hopes of speeding up the growth and regeneration processes. Specifically, we asked whether soil, light, and temperature affect the germination success of *V. adunca* seeds. We included small pH differences as part of our soil treatment because fire is currently being used in *V. adunca* habi-

tat restoration on the west coast (Pickering et al. 2000) and pH can change following fire. The importance of pH for seed germination is generally species dependent; some species are strongly affected, while others, not at all (Perez-Fernandez et al. 2006).

MATERIALS AND METHODS

Study Species

Viola adunca (J.E. Smith) is a temperate, herbaceous, perennial plant growing to 15 cm. The Missouri Botanical Garden lists 15 variants or subspecies; the most widespread is *V. adunca* var. *adunca*, which is found from California to New England (National Plant Data Center: http://plants.usda.gov/cgi_bin/topics.cgi?earl=plant_profile.cgi&andsymbol=VIAD).

It is known by several common names, including early-blue, dogs-tooth, hook-spur and sand violet. *Viola adunca* is widespread throughout North America, ranging from Greenland to Alaska, and New York to California (Gleason and Cronquist 1991). In the upper midwest, *V. adunca* occurs in moist meadows, prairies, open ground, moist to dry woods, and gravely, sandy soil (Hitchcock and Cronquist 1973; Voss 1985; UWSP Herbarium website: <http://wisplants.uwsp.edu/WisPlants.html>).

Experimental Methods

Some seeds were collected in the field during the growing season of 2004; other seed was obtained from the Oregon Zoo. Seeds were randomized prior to treatment. We conducted two separate germination trials: the first set of seeds was planted on October 29th, 2004; the second set between

TABLE 1. Treatments used in the first germination trial. Four treatments were used for soil and four treatments for light/temperature regime, giving a 4X4 factorial experiment with 16 total treatments. "Potting soil" was the sphagnum-based Berger's BM1 brand without fertilizer; "Soil mix" consisted of 40% Pro-Mix Potting Soil, 40% sand, and 20% Fox Farm Planting Mix. All pots were stratified in darkness at 3.9°C for two weeks. *Treatments listed below follow the initial two weeks of stratification.* After the treatment listed, pots were moved to the greenhouse.

TREATMENTS	
Number	Soil Light/Temperature
1.	Control: potting soil; greenhouse light and temperature regime.
2.	Potting soil; two weeks in dark at 7.2°C, then greenhouse.
3.	Potting soil; two weeks at 10 hours light:14 hours dark, 7.2°C, then greenhouse.
4.	Potting soil; two weeks at 10 hours light:14 hours dark, 7.2°C; two weeks at 12 hours light:12 hours dark, 10.6°C; then greenhouse.
5.	Potting soil with the pH lowered; greenhouse light and temperature regime.
6.	Potting soil with the pH lowered; two weeks in dark at 7.2°C, then greenhouse.
7.	Potting soil with the pH lowered; two weeks at 10 hours light:14 hours dark, 7.2°C, then greenhouse.
8.	Potting soil with the pH lowered; two weeks at 10 hours light:14 hours dark, 7.2°C; two weeks at 12 hours light:12 hours dark, 10.6°C; then greenhouse.
9.	Soil mix; greenhouse light and temperature regime.
10.	Soil mix; two weeks in dark at 7.2°C, then greenhouse.
11.	Soil mix; two weeks at 10 hours light:14 hours dark, 7.2°C, then greenhouse.
12.	Soil mix; two weeks at 10 hours light:14 hours dark, 7.2°C; two weeks at 12 hours light:12 hours dark, 10.6°C; then greenhouse.
13.	Soil mix with the pH lowered; greenhouse light and temperature regime, then greenhouse.
14.	Soil mix with the pH lowered; two weeks in dark at 7.2°C, then greenhouse.
15.	Soil mix with the pH lowered; two weeks at 10 hours light:14 hours dark, 7.2°C, then greenhouse.
16.	Soil mix with the pH lowered; two weeks at 10 hours light:14 hours dark, 7.2°C; two weeks at 12 hours light:12 hours dark, 10.6°C; then greenhouse.

January 28th and February 9th, 2005. For the first trial, ten seeds each were sown in 10 cm × 10 cm pots and watered from above with reverse osmosis (RO) water, with eight replicates per treatment (Table 1). After seeds were sown, all flats were placed in a growth chamber for cold stratification at 3.9°C in darkness. After two weeks, flats were separated into light/temperature regimes (Table 1). Soil was kept moist with RO water throughout the treatments. Flats were checked every other day for germination. After initial light/temperature treatments, pots were moved to the greenhouse and randomized throughout the flats. Mean temperatures in the greenhouse varied from 19.6°–27°C during the day, and 14.6°–20.1°C at night. This trial ended on February 2nd, 2005. All seedlings in each treatment were erroneously combined before data analysis, so during analysis we counted each treatment as a single replicate.

To determine whether pH affected germination, pH was decreased from 5.5–6.0 to 4.5–5.0 in treatments 5–8 and 13–16 by mixing ferrous sulfate (FeSO₄) powder directly into moistened soil. The pH of the soil mixture was tested with Hydriion Paper pH strips (Micro Essential Laboratories).

We ran a second germination trial to more clearly test light and temperature requirements for germination. Seeds were sown for the second trial as in Trial 1 with eight replicates per treatment (Table 2); however, control treatments were not subjected to a cold stratification period as they had been in Trial 1. All treatments were planted in COIR-based, Scotts Metro-Mix 336P potting mix. One week into the experiment one of the growth chambers malfunctioned, allowing the temperature to increase to 32.8°C for two days. After discovering the problem, we replanted all treatments except the controls that did not undergo cold stratification (Treatments 1 and 2). We kept the malfunctioned treatments in the growth chambers along with the newly planted treatments, so these two treatments (7 and 8) had 16 replicates rather than eight. Final count of seedlings occurred on April 25th, 2005.

Mean daily greenhouse temperature was 23.2°C during the first trial and 24.0°C in the second trial. Night temperature averaged 16.5°C in the first trial, 18.0°C in the second trial. Though not large in magnitude, the temperature differences in the greenhouse during the two germination trials were significant (Two-Sample t-test; $p < 0.05$).

Data Analysis

Because replicates in Trial 1 were accidentally combined before data analysis, we analyzed each treatment type (soil, light/temperature) separately using Analysis of Variance (ANOVA) tests; thus

TABLE 2. Treatments used in the second germination trial. Soil type was controlled, with eight variants of temperature and light. The first two treatments started in the greenhouse (no cold stratification). All other treatments were placed in the greenhouse after treatment in the growth chambers.

Treatment Number	Treatment	Light/Temperature Regime
1	Control	Greenhouse
2	Temperature Control	Greenhouse, with two weeks dark
3	Single Temperature Fluctuation with Light	2 weeks @ 12 hours light:12 hours dark; 3.9°C
4	Single Temperature Fluctuation with Dark	2 weeks dark; 3.9°C
5	Multiple Temperature Fluctuations with Light	6 weeks @12 hours light:12 hours dark; 2 weeks each @ 3.9°C, 7.2°C, 3.9°C
6	Multiple Temperature Fluctuations with Dark	6 weeks dark; 2 weeks each @ 3.9°C, 7.2°C, 3.9°C
7	Unplanned Temperature Fluctuation with Light	7 weeks @ 12 hours light: 12 hours dark; 1 week @ 3.9°C, 2 days @ 32.8°C, 2 weeks each @ 3.9°C, 7.2°C, 3.9°C
8	Unplanned Temperature Fluctuation with Dark	7 weeks dark; 1 week @ 3.9°C, 2 days @ 32.8°C, 2 weeks each @ 3.9°C, 7.2°C, 3.9°C

each soil treatment acted as a replicate for the light/temperature test, and vice versa. With the lack of replication we were unable to test for interactions; thus we made the assumption that the two treatments did not interact. Data from the second germination trial failed to meet the parametric test assumptions of normality and equal variance even with transformations, so they were analyzed using the non-parametric Kruskal-Wallis test. All statistical tests were performed in Systat 11.0 (Systat 2004).

RESULTS

Soil treatments (soil type and pH) in our first germination trial yielded significantly different percentages of germination ($p < 0.001$; $df = 1$; Two-Factor ANOVA) (Fig. 1). There was also a significant interaction between soil type and pH ($p < 0.001$; $df = 1$). Highest percentages of germination were achieved in the unaltered pH, BM1 potting soil. Light/temperature treatments did not differ significantly from one another in the first trial ($p = 0.95$; $df = 3$; Single-Factor ANOVA) (Fig. 2). Mean germination for the entire trial was 15%.

In the second germination trial, overall germination was lower, at 10.75%. Both light and temperature treatments significantly affected germination percentage ($p < 0.001$; $df = 1, 2$ for light and temperature, respectively; Kruskal-Wallis; Fig. 3). Pairwise comparisons showed that the strong difference in temperature treatments was due to the high germination in Treatment 7, seeds that underwent a dramatic, unscheduled fluctuation in temperature in the growth

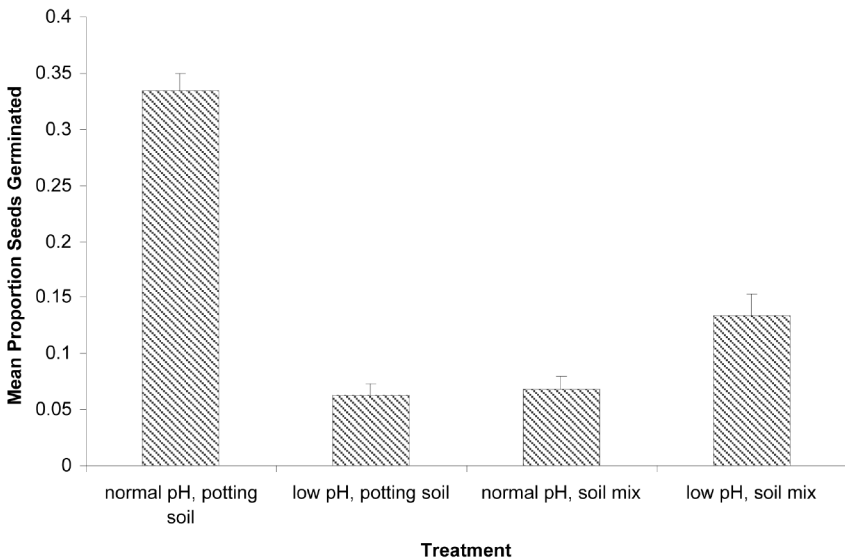


FIGURE 1. The effect of soil type and pH on proportion of seeds germinated in *V. adunca*, Trial 1. Both pH and soil type had a significant impact on germination, and there was a significant interaction effect between the two soil properties. Error bars represent ± 1 standard error of the mean.

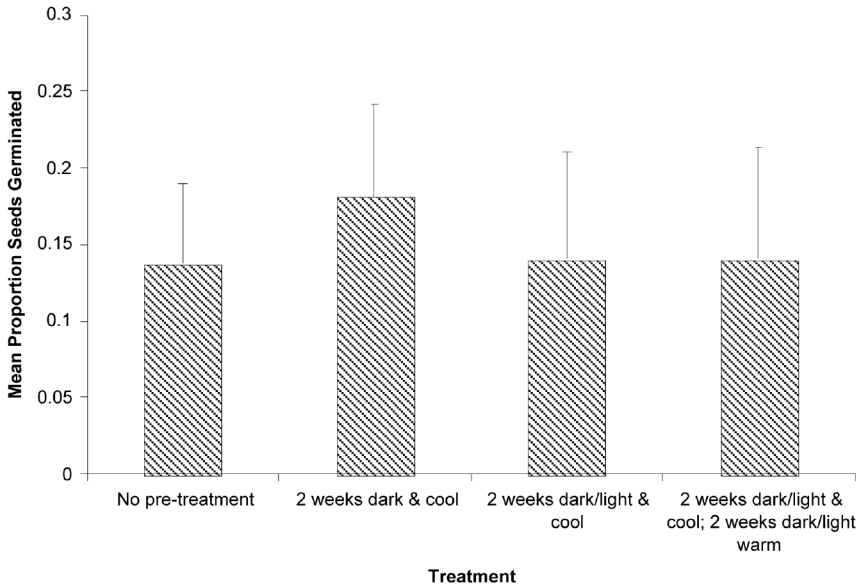


FIGURE 2. The effect of light/temperature treatments on proportion of seeds germinated in *V. adunca*, Trial 1. There were no significant effects of this treatment in Trial 1. Error bars represent ± 1 standard error of the mean.

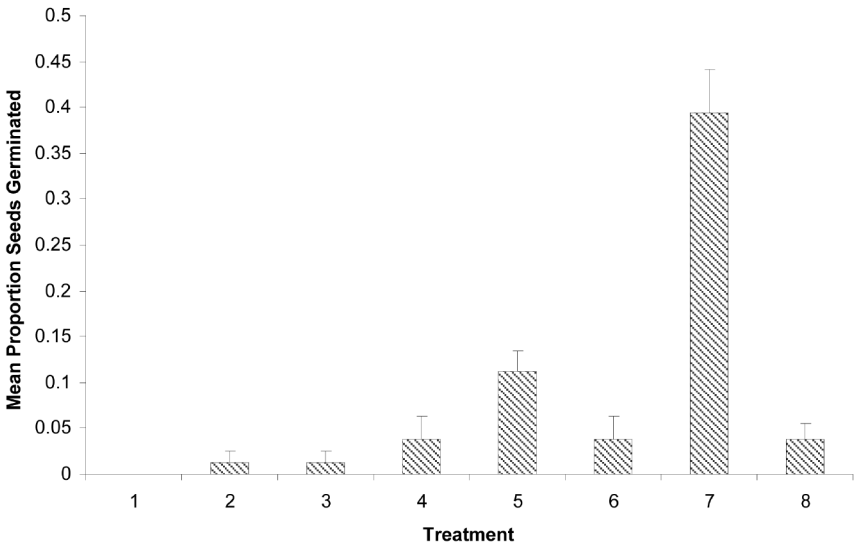


FIGURE 3. Mean proportion of plants germinated per replicate of each treatment in Trial 2. Treatment 7 differed significantly from all others ($p < 0.001$; Kruskal-Wallis). Error bars represent ± 1 standard error of the mean.

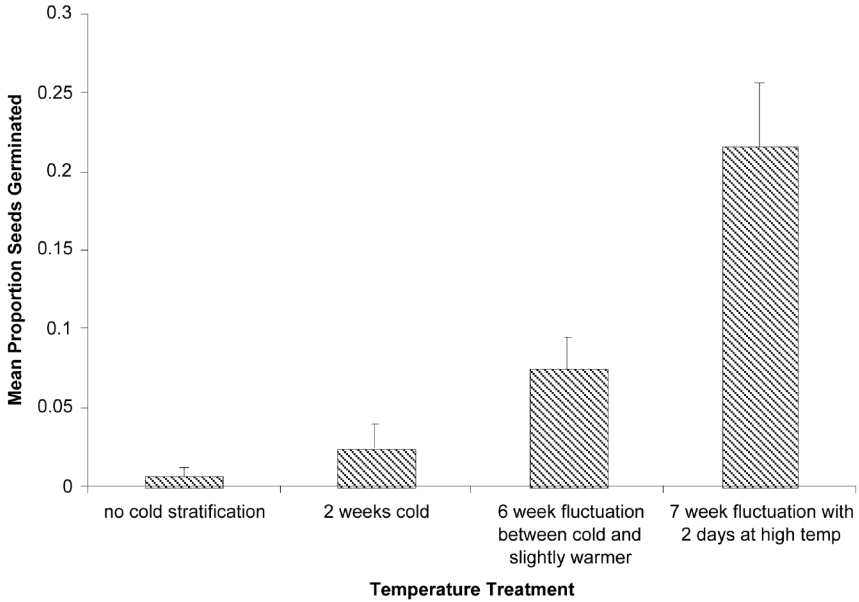


FIGURE 4. The effect of temperature treatments on proportion of plants germinated in Trial 2. The first bar represents replicates with no period of cold stratification (Treatments 1 & 2); the second bar represents replicates with a two-week period of cold stratification (Treatments 3 & 4); the third bar represents replicates with a planned temperature fluctuation of cold, warmer, cold, then greenhouse (Treatments 5 & 6); and the fourth bar represents replicates with the unplanned high temperature fluctuation (Treatments 7 & 8), along with a planned fluctuation as was done in Treatments 5 & 6. Error bars represent ± 1 standard error of the mean.

chamber (Figure 4). Germination in this treatment differed significantly from all other treatments ($p < 0.001$; Bonferroni Adjustment) while germination in other treatments did not differ from one another. We removed Treatments 7 and 8 from the analysis to see whether the planned fluctuation affected germination. Germination percentage did increase significantly ($p = 0.004$; Kruskal-Wallis) when seeds were subjected to a relatively small temperature increase, then a decrease, in treatments 5 and 6. Seeds that did not have a period of constant darkness exhibited higher germination success ($p < 0.001$; Kruskal-Wallis; Fig. 5); length of the constant dark period did not affect the proportion of seeds that germinated ($p > 0.05$; Bonferroni Adjustment, Pairwise Comparisons).

DISCUSSION

In spite of the challenges and problems encountered in this project, the two germination trials yielded interesting and potentially useful results. Soil type, pH, light and temperature all affected *V. adunca* germination. Highest germina-

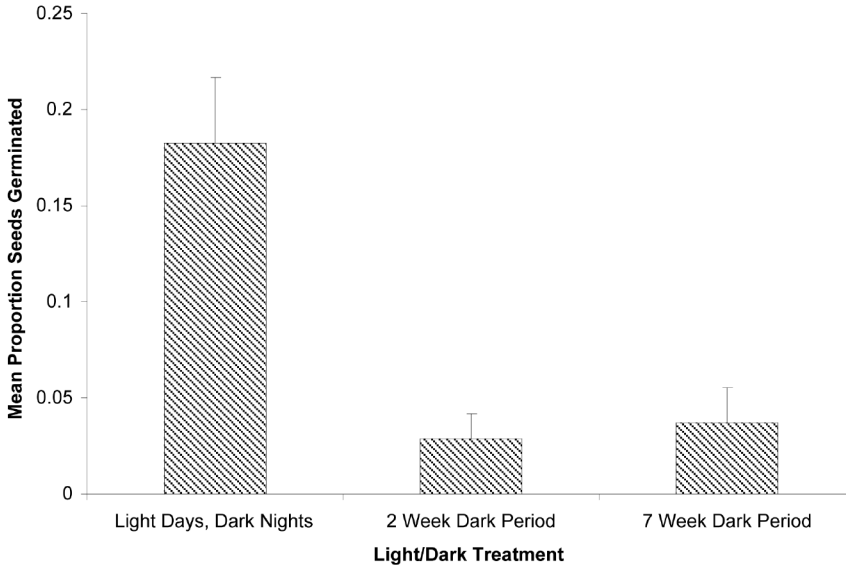


FIGURE 5. The effect of light treatment on proportion of *V. adunca* seeds germinated in Trial 2. The seeds represented in the first bar were subjected to light days and dark nights during the entire experiment (Treatments 1, 3, 5, 7). The seeds represented by the second bar underwent a two week period of constant darkness prior to being placed in the greenhouse (Treatments 2 & 4). The final bar represents seeds that underwent a 6–7 week period of constant darkness prior to being placed in the greenhouse (Treatments 6 & 8). Seeds treated to alternating light-dark periods germinated at a significantly higher rate than seeds treated to constant darkness. Error bars represent ± 1 standard error of the mean.

tion percentages occurred during the first germination trial, and seeds appeared to respond best to planting in straight potting soil. That there was a significant interaction between soil type and pH bolsters the result that soil is important in germination success, and suggests that further research is warranted to tease apart differences in the soil mixtures and the interaction between pH and soil type. Higher germination percentages in Trial 1 could have been due in part to the fact that in Trial 1, even the control plants underwent a period of cold stratification. Alternatively, soil mixture may simply be the most important factor for *V. adunca* germination success.

Slightly higher pH values resulted in higher germination success. Upon reflection, this result was not surprising. Prescribed burns on the Oregon coast have resulted in higher germination in *V. adunca* (Pickering et al. 2000) and when burns change soil pH, generally the result is an increased pH (Rhoades et al. 2004; Murphy et al. 2006). In west coast forests, *V. adunca* is generally found in meadows rather than amongst trees. Its habitat is early successional; eventually coniferous forests colonize the meadows (USFWS 2001). Disturbances such as fire create a mosaic of meadow habitat that can be colonized by *V. adunca* and

other herbs. Thus it is likely that *V. adunca* is less well adapted to the lower pH values typical of a coniferous forest.

We suspect that the negative result of light/temperature treatment in the first trial may have been an anomaly, either due to the small sample size from the aforementioned error, or because all seeds in the first trial underwent an initial cold stratification period, which may have been sufficient for germination of those seeds.

During the second germination trial we controlled for soil type and pH to more clearly elucidate the relationship between germination and light/temperature. The most striking result was the significantly higher germination percentages with the inadvertent fluctuation in temperature (in the malfunctioning growth chamber). This suggests that fluctuating temperatures, which can occur during seasonal change, may result in higher percentages of germination. Doohan et al. (1991) showed that in a controlled environment, *V. arvensis* germination was highly favored by cool, diurnally fluctuating temperatures, while reduced germination in the field was attributed to an induction of dormancy by high soil temperatures. Alternation of temperature, while not important for germination of many species (Ghersa et al. 1992; Jones et al. 2004; Leon et al. 2004), enhances germination in others (Cochrane et al. 2002; Benvenuti et al. 2004; Leon et al. 2004). It may be that amplitude of temperature alternation is more important than actual temperatures (Leon et al. 2004). In our experiment, a single temperature fluctuation with very high amplitude appears to have sufficiently increased number of seeds germinating. Other germination research suggests that temperature and temperature fluctuation may be the most important cues for seeds to germinate (Baskin and Baskin 1998).

Light requirements can vary between species (Baskin and Baskin 1985) and even within a species depending on the age of the seed (Baskin and Baskin 1995). Other researchers have found that a dark period can enhance *Viola* germination (Doohan et al. 1991). *Viola arvensis* appears to prefer light periods such as would be found in disturbed sites (Baskin and Baskin 1995). Short periods of darkness did not increase germination percentage in *V. adunca* and our data suggest that an extended period without light inhibits germination in this species. A dark period has been found to be relatively unimportant in other studies as well (Burgess et al. 2002).

Overall, soil mixture and temperature fluctuation appeared to influence *V. adunca* seed germination more than the other factors we tested. Our results suggest several areas of future research. For example, further experimentation on the interplay between soil type and pH would help us understand the conditions of each factor that are preferred by *V. adunca*. A comparison of *in situ* conditions vs. controlled growth chamber/greenhouse germination would help to determine a more precise light and temperature regime. Fluctuating diurnal temperatures as well as a gradual increase of temperature over time to simulate seasonal changes could facilitate greater understanding of the germination requirements for this species. Such experiments help us understand of specific life history strategies, and have the potential to improve the efficiency and success of restoration and management.

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