

**Tolerance range for dormancy loss and germination differs among  
dispersal strategies**

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A Thesis Presented to the Graduate Faculty of Middle Tennessee State University in  
Partial Fulfillment of the Requirements for the Degree Master of Science in Biology

Middle Tennessee State University

August 2016

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## ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere gratitude to my supervisor Dr. Jeffrey L. Walck, for his patience, guidance and continuous support throughout my thesis work. He was a great mentor who helped me overcome the challenges of my research and mature as a professional scholar and research scientist. It would not be possible for me to complete my degree without his kind help.

I'm deeply grateful to my committee members, Dr. Siti N. Hidayati and Dr. R. Stephen Howard for their support and encouragement. Their suggestions immensely helped make my thesis and research project a success.

I would like to thank, Dr. Kayri Havens and Jessa Finch (Chicago Botanic Garden) for collecting seeds, coordinating the collection efforts by other people, and contributing their ideas to the project. I'm thankful to Eric Limbard, Rachael Hicks, Mariam Boutros and other undergraduate students of Dr. J. Walck's lab who helped me set up experiments and collect data.

I would like to thank my parents and my family members for their love and support throughout my life. Besides all, I'm grateful to my loving and caring husband Thilina Ruwan Fernando for his encouragement and being with me in every single step of this journey.

## ABSTRACT

Germination has an optimum temperature and minimum and maximum thresholds [hereafter, tolerance range (TR)]. I reasoned that species with limited dispersal would be more specialized to local conditions and have narrower TR than those with wide dispersal. Four species contrasting in dispersal strategies were studied: *Penstemon digitalis*, *P. tubaeiflorus* (gravity), *Physalis longifolia* (animal) and *Asclepias syriaca* (wind). I hypothesized that TR would differ (narrowest to widest): *Penstemon* < *Physalis* < *Asclepias*. Seeds were collected from MO to MN; stratified at 1, 5, and 9°C; and incubated at 15/6, 20/10, 25/15, and 30/15°C. Levins' B was calculated; values approaching 1 vs. 0 represent a wide vs. narrow TR. *Asclepias* and *Physalis* seeds germinated highest at 20/10-30/15°C regardless of stratification temperature, and *Penstemon* seeds at 15/6-30/15°C following 1°C stratification. TRs for cold-stratified seeds were: *Penstemon* < *Physalis* < *Asclepias*. Thus, my hypothesis was supported that TR varied according to dispersal strategy.

## TABLE OF CONTENTS

	Page
LIST OF TABLES.....	vi
LIST OF FIGURES.....	vii
CHAPTER	
I. INTRODUCTION.....	1
II. MATERIALS AND METHOD.....	6
STUDY SPECIES.....	6
SEED COLLECTION AND MASS.....	6
GENERAL GERMINATION PROCEDURES.....	7
GERMINATION TESTS.....	8
TEMPERATURE TOLERANCE RANGE.....	9
SEQUENCE OF TEMPERATURE REGIMES.....	9
STATISTICAL ANALYSES.....	10
III. RESULTS.....	13
SEED MASS.....	13
GERMINATION OF NON-STRATIFIED SEEDS.....	13
GERMINATION OF STRATIFIED SEEDS.....	14
TEMPERATURE TOLERANCE RANGES.....	15
SEQUENCE OF TEMPERATUER REGIMES.....	15

IV. DISCUSSION.....	30
LITERATURE CITED.....	37

## LIST OF TABLES

<b>Tables</b>	<b>Page</b>
1. Locations for seed collections of the study species during autumn 2013	11
2. Temperature sequences through which fresh seeds of the study species were moved to simulate temperatures from dispersal to germination	12
3. Seed mass of the study species and populations	17

## LIST OF FIGURES

Figures	Page
1. Germination (mean $\pm$ SE) across temperatures for fresh seeds	18
2. Germination (mean $\pm$ SE) across temperatures for control seeds	19
3. Germination (mean $\pm$ SE) for stratified seeds of <i>Penstemon digitalis</i> from IL	20
4. Germination (mean $\pm$ SE) for stratified seeds of <i>Penstemon tubaefflorus</i> from MO	21
5. Germination (mean $\pm$ SE) for stratified seeds of <i>Physalis longifolia</i> var. <i>subglabrata</i> from WI	22
6. Germination (mean $\pm$ SE) for stratified seeds of <i>Physalis longifolia</i> var. <i>subglabrata</i> from MN	23
7. Germination (mean $\pm$ SE) for stratified seeds of <i>Asclepias syriaca</i> from MO	24
8. Germination (mean $\pm$ SE) for stratified seeds of <i>Asclepias syriaca</i> from IL	25
9. Germination (mean $\pm$ SE) for stratified seeds of <i>Asclepias syriaca</i> from MN	26
10. Temperature tolerance ranges (mean $\pm$ SE), measured as Levins' B, for fresh seeds from three genera differing in dispersal strategies	27
11. Temperature tolerance ranges (mean $\pm$ SE), measured as Levins' B, for 12-week cold-stratified seeds	28

12. Germination (mean  $\pm$  SE) of seeds moved through a series of temperatures

29



## CHAPTER I

### INTRODUCTION

Climate change is having a profound effect on distributions of species, resulting in rapid shifts of ranges poleward and higher elevations (Walther *et al.*, 2002; Parmesan, 2006; Walker *et al.*, 2006; Vitt *et al.*, 2009; Valladares *et al.*, 2014). The mechanisms enabling species' responses to climate change occurs in the early life stages of the plant life cycle, such as seed dormancy, germination and seedling establishment. These aspects are critical stages for plant regeneration, persistence and distribution (Walck *et al.*, 2011; De Frenne *et al.*, 2011; Valladares *et al.*, 2014). Different life history strategies, such as in seed dispersal, contribute to the interspecific and intraspecific variation in seed dormancy and germination, which ultimately determine species' responses to spatial and temporal variation of environmental cues such as temperature (Horvitz and Schemske, 1994; Coops and Vedle, 1995). For instance, long distance dispersal mechanisms are considered beneficial for the persistence of species in their ecological ranges under a warming climate (Cain *et al.*, 2000; Nathan *et al.*, 2002; Vitt *et al.*, 2009). However, the interaction between life history traits, like seed dispersal, and the tolerance range for germination is poorly understood.

Dispersal is the movement of seeds (propagules) away from the parent plant to new locations for the establishment of the next generation (Nathan and Muller-Landau, 2000; Wang and Smith, 2002; Nathan *et al.*, 2008). Seed dispersal mechanisms are mediated by abiotic and biotic vectors such as wind, water and animals, respectively (Hawe and Smallwood, 1982; Chamber and MacMahon, 1994). Plant species with

specialized dispersal mechanisms exhibit specific phenotypic traits which are predicted as adaptations for vector mediated dispersal (Howe and Smallwood, 1982). For instance, wind dispersed species (e.g., *Asclepias syriaca*) have wings, plumes and produce relatively small, light seeds (Bhowmik and Bandeen, 1976; Meyer *et al.*, 1995). In contrast, animal dispersed seeds (e.g., *Physalis longifolia*) have fleshy nutritive parts such as aril and pericarp (Kindscher, 2012) and produce chemical attractants (Howe and Smallwood, 1982). On the other hand, there are species whose seeds are gravity (or passively) dispersed (e.g., *Pestemon digitalis*). After the dispersal of fully ripened (fresh) seeds, seeds of some species experience a period of dormancy (Baskin and Baskin, 1998). This period prevents germination during adverse conditions for seedling establishment, and physio-biochemical changes inside the seeds delay germination until conditions are more favorable (Baskin and Baskin, 1998).

Seed dormancy and germination are key physiological processes that determine the expression of post-germination traits of plants and, consequently, affect the ecological niche and geographic distribution of species (Brandle *et al.*, 2003; Donohue *et al.*, 2010). These traits are controlled by multiple genes and the expression of which is influenced by environmental factors such as temperature, moisture, oxygen content and light (Baskin and Baskin, 1998; Koornneef *et al.*, 2002; Donohue *et al.*, 2005; Finch-Savage and Leubner-Metzger, 2006). Temperature is considered the key environmental cue that regulates germination in seeds with a physiological-basis for dormancy loss (Baskin and Baskin, 1998; Probert, 2000; Bradford, 2002; Baskin and Baskin, 2004; Finch-Savage and Leubner-Metzger, 2006). Physiological dormancy can be overcome by a period of moist, low-temperature (1-10°C) conditions (cold stratification) or by a period of dry or

moist, high-temperature ( $>15^{\circ}\text{C}$ ) conditions (after-ripening or warm stratification). However, the duration of the dormancy loss period and the specific conditions (like temperature) under which it occur are species dependent (Farmer *et al.*, 1986; Walck *et al.*, 2000; Baskin and Baskin, 2004). For instance, a study conducted with 135 populations of 38 *Penstemon* species ranging from warm desert to alpine tundra showed that populations from habitats with severe winters require a long period of cold stratification, whereas populations from habitats with mild winters require a short period of cold stratification (Meyer *et al.*, 1995).

Dormancy break and germination of seeds have an optimum temperature condition ( $T_{\text{opt}}$ ) and minimum ( $T_{\text{min}}$ ) and maximum ( $T_{\text{max}}$ ) thresholds (hereafter, tolerance range), which is the range of temperatures under which these physiological processes take place (Cave *et al.*, 2011; Orru *et al.*, 2012; Sheldon and Tewksbury, 2014). For instance, the temperature tolerance range for germination of *Calandrinia* sp. has been reported as:  $T_{\text{min}}$  or the base temperature as  $5.8 - 7.9^{\circ}\text{C}$ ,  $T_{\text{max}}$  as  $22.5^{\circ}\text{C}$  and  $T_{\text{opt}}$  as  $47.2^{\circ}\text{C}$  (Cave *et al.*, 2011). Responses of species to climate change (and other environmental disturbances) are highly dependent on the tolerance range, since it determines whether plants may persist in their current distribution, migrate to track climates or adapt to new conditions, or go extinct (Donohue *et al.*, 2005; Donohue *et al.*, 2010; reviewed by Buckley *et al.*, 2010).

Variation in the tolerance range within and among species may be influenced by the (genetic) neighborhood size, i.e. the size of an area from which the parent may be assumed to be drawn at random, and neighborhood size is mediated by dispersal strategy (Wright, 1943; Kawata, 1996). According to Wright, the neighborhood size ( $N_e = 4\pi\delta\sigma^2$ )

is proportional to the variance of the dispersal distance ( $\sigma^2$ ) and density of the population ( $\delta$ ) (Wright, 1943; Sacchi, 1987; Kawata, 1996). Furthermore, species with seed dispersal mechanisms have the opportunity to utilize a broad range of resources and thus have a broad niche breadth (Feinsinger *et al.*, 1981). Hence, if the niche breadth is defined in terms of germination temperature, species that are widely dispersed (e.g. by wind) should break dormancy and germinate under broad range of temperatures. In contrast, seeds of species that are narrowly dispersed (e.g. by gravity) are locally adapted and presumably have a small neighborhood size and narrow tolerance range. It is known that temperature requirements for germination differ among populations of a species (Brandle *et al.*, 2003; De Frenne *et al.*, 2012). For example, the thermal thresholds for germination differed between the low and high altitude populations of *Vitis vinifera* subsp. *sylvestris* (Orru *et al.*, 2012). However, tolerance ranges have been less studied as compared to temperature requirements and traits that may influence tolerance ranges (such as dispersal) have been rarely investigated.

I predicted that the temperature tolerance range would vary among species differing in dispersal strategies in the following order (narrowest to widest): gravity < animal < wind. To test my hypothesis, I determined tolerance ranges of both fresh and cold-stratified seeds of species with these dispersal strategies. Fresh seeds were incubated across a range of temperatures approximating growing season conditions. Seeds were cold stratified for various durations under a range of temperatures and then incubated across the same growing season temperature range. Lastly, I investigated the relationship between tolerance range and the simulated timing of germination in nature. I hypothesized that species with wide tolerance range may shift the timing of germination

to autumn whereas species with narrow tolerance range would not such that the timing of germination would remain in spring. To test this predictions, fresh seeds were moved through a sequence of temperatures simulating current and future temperatures starting at autumn conditions (time of dispersal) and ending at spring conditions (time of germination).

## CHAPTER II

### MATERIALS AND METHOD

**Study species:** Four species that occur in mostly open habitats (e.g. old fields, meadows, along roadsides, prairies, open woods; Gleason and Cronquist, 1991) and which differ in dispersal syndromes were selected for the study. *Penstemon digitalis* Nutt. ex Sims (Foxglove beardtongue; Plantaginaceae) is a herbaceous perennial that is found in northeastern to southcentral United States, and *P. tubaeiflorus* Nutt. (White wand beardtongue) is restricted to Ozark region of the United States (Kartesz, 2015; hereafter, referred to as *Penstemon*). Seeds of both *Penstemon* species are gravity dispersed. *Physalis longifolia* Nutt. var. *subglabrata* (Mack. & Bush) Cronquist (Longleaf groundcherry; Solanaceae; hereafter, referred to as *Physalis*) is a perennial herb that produces a berry like fruit. The seeds are dispersed by animals facilitated by the presence of fleshy edible pericarp of the fruit (Kindscher *et al.*, 2012). This species occurs from northeastern to central United States (Kartesz, 2015). *Asclepias syriaca* L. (Common milkweed; Apocynaceae; hereafter, referred to as *Asclepias*) occurs is a herbaceous perennial with a wide distribution in northcentral and northeastern United States (Kartesz, 2015). The fruit is a simple follicle and seeds have plumes that enable wind dispersal (Bhowmik, 1976; Sacchi, 1987).

**Seed collection and mass:** Seed collection of the four species was coordinated by the Chicago Botanic Garden (Glencoe, IL) during autumn 2013 (Table 1). Staff at the Chicago Botanic Garden cleaned the seeds (e.g. by removing them from capsules or by

removing plumes), and then the seeds were mailed to Middle Tennessee State University (Murfreesboro, TN), where all laboratory experiments were conducted. The seeds were counted into lots of 25 each and stored dry at room temperature (21°C) in paper envelopes until experiments started in early December 2013. Fifteen (or 8 for *Asclepias* from Nachusa Grasslands) sets of 25 seeds each were weighed to the nearest 0.0001 g.

**General germination procedures:** Seeds were placed in 6 cm diameter plastic Petri dishes filled with swimming pool filter sand. The sand was moistened with distilled water to saturation before seeds were added to the dishes. Twenty five seeds were placed in each Petri dish and three replicates (i.e. three dishes) were used for each treatment. The three Petri dishes for a replicate were stacked together, with the top, middle, and bottom dish designated as dish 1, 2, and 3, respectively. To reduce moisture loss, the stack of dishes was sealed with plastic wrap and sand in each dish was moistened with distilled water as required throughout the experiment.

All germination experiments were conducted in temperature- and light-controlled incubators. Three incubators were set to constant temperatures of 1, 5 and 9°C, and four other incubators to 12/12 h daily alternating temperature regimes of 15/6, 20/10, 25/15, and 30/15°C. The constant temperatures represented cold stratification (winter) conditions and the alternating temperatures represented growing season conditions across eastern United States. The daily photoperiod in the incubators was 12 h, simulating the approximate day length at all seed collection sites. Cool white 20 W fluorescent tubes, which produced a photosynthetic photon flux density (400–700 nm) at seed level of ca.  $40 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , were used as the light source.

Protrusion of the radicle ( $> 2$  mm) from the seed was the criterion for the occurrence of germination. Seeds were checked for germination at 2-week intervals; checking of seeds was done under a dissecting microscope for the small-seeded *Penstemon* and *Physalis*. At the end of the germination tests, the viability of the ungerminated seeds was determined using the pinch test: the seeds were pinched with forceps under a dissecting microscope and white, firm embryos were considered viable and brown, soft embryos were non-viable. The standard tetrazolium test confirmed the viability of the white embryos and non-viability of the brown embryos. Most seeds with the presence of fungi were non-viable. Germination percentage was calculated based on the number of viable seeds.

**Germination tests:** Non-stratified seeds were incubated at all temperatures and checked for germination at 2-week intervals for a total of 22 weeks; seeds were considered as “fresh” during the first 2 weeks of incubation and as “control” for the 22 weeks. At the end of 22 weeks, viability of seeds was determined. Stratified seeds were placed at 1, 5 and 9°C for 4, 8, 12, 16 and 20 weeks and then following each period they were incubated at 15/6, 20/10, 25/15 and 30/15°C for 2 weeks. The lengths of cold stratification about equaled the lengths of winter across the distribution ranges for populations of the study species. Follow the 2 week incubation period, the number of germinated and non-germinated but viable seeds were determined.



**Temperature tolerance range:** Tolerance ranges (TR) for fresh seeds and 12-week stratified seeds were calculated using Levins' B equation (Levins, 1968; Feinsinger *et al.*, 1981).

$$TR = 1/(R \sum_i p_i^2),$$

where  $p_i$  is the proportion of germination in the *ith* condition and R is the number of temperatures tested. R equaled seven (1, 5, 9, 15/6, 20/10, 25/15, and 30/15°C) and twelve (four alternating temperatures following stratification at 1, 5, and 9°C) for fresh and stratified seeds, respectively. This measure ranges from 0 to 1.0: values approaching 1.0 represent wide TR and those approaching 0 represent narrow TR. A separate TR was calculated for each dish in the stack across the temperature range, i.e. a TR was determined for each dish (replicate) in the stack (top, middle, bottom) across the temperature range.

**Sequence of temperature regimes:** Fresh seeds were passed through a sequence of monthly temperatures (a “move-along” design) simulating the time between dispersal (October and November) through dormancy loss (December to February) to germination (March). Temperatures in the sequence matched those in the location of the population from which seeds were collected, and they represented simulated future temperatures (i.e. an approximate +5°C by the year 2080 under the B1 emissions scenario from MN to MO, Girvetz *et al.*, 2009) and current or present-day temperatures (i.e. temperatures recorded at the nearest weather station of the population and averaged between 1981 and 2010 without the +5°C; National Oceanic and Atmospheric Administration, 2015) (Table 2).

**Statistical analyses:** A one-way analysis of variance (ANOVA) or t-test was used to examine seed mass among or between populations of *Physalis* or *Asclepias*; variances were homoscedastic for both analyses (SPSS, 2012). Germination percentages were arcsin-square root transformed for analyses to correct for non-normality of the data. For non-stratified seeds, temperature was the factor in ANOVAs for both *Penstemon* species and temperature and population were factors for ANOVAs on *Asclepias* and *Physalis*. For stratified seeds, stratification temperature and length and incubation temperature were factors in ANOVAs for both *Penstemon* species and stratification temperature and length, incubation temperature, and population were factors for ANOVAs on *Asclepias* and *Physalis*. TRs were calculated for fresh and cold-stratified seeds from each population classified as to the dispersal strategy for the genus (gravity, animal, wind). A t-test or one-way ANOVA was performed to test if TR differed between or among populations of *Physalis* or *Asclepias*, respectively. Then, a one-way ANOVA followed by protected least significant difference tests (PLSD,  $P = 0.05$ ) was performed to test whether TR differed among the dispersal strategies. A repeated-measures ANOVA was used to examine the sequence of temperature experiment, with time (through the sequence) as the within-subject effect, and condition (current vs. future temperature regime) and/or population as between-subject effects. Greenhouse–Geisser corrected probabilities are reported.

Table 1. Locations for seed collections of the study species during autumn 2013.

Species	State	Location	Latitude	Longitude
<i>Penstemon digitalis</i>	IL	Nachusa Grasslands	41.889941	-89.347313
<i>Penstemon tubaefflorus</i>	MO	Tingler Prairie Conservation Area	36.610783	-91.875700
<i>Physalis longifolia</i> var. <i>subglabrata</i>	MN	MN Landscape Arboretum, Bennett-Johnson Prairie	44.859539	-93.625623
	WI	Lone Rock Prairie	42.685819	-89.231736
<i>Asclepias syriaca</i>	MO	Along west Outer Road just west of the intersection of I-44 and MO Hwy 141	38.538370	-90.502090
	IL	Nachusa Grasslands	41.880805	-89.341641
	MN	MN Landscape Arboretum, Bennett-Johnson Prairie	44.859539	-93.625623

Table 2. Temperature sequences through which fresh seeds of the study species were moved to simulate temperatures from dispersal to germination.

Month	Part of life cycle for seeds	MO		IL/WI		MN	
		Current	Future	Current	Future	Current	Future
October	Dispersal	20/10	25/15	20/10	25/15	15/6	20/10
November	Dispersal	15/6	20/10	15/6	20/10	1	15/6
December	Dormancy loss	1	5	1	5	1	5
January	Dormancy loss	1	5	1	5	1	5
February	Dormancy loss	1	5	1	5	1	5
March	Germination	15/6	20/10	1	15/6	1	15/6

## CHAPTER III

### RESULTS

**Seed mass:** Seed mass differed significantly between populations of *Physalis* ( $P = 0.008$ ) and among populations for *Asclepias* ( $P \leq 0.0001$ ). The hierarchy of seed sizes based on mass were: *Asclepias* > *Physalis* > *Penstemon* (Table 3).

**Germination of non-stratified seeds:** Germination for fresh seeds of the four species differed significantly across the range of temperatures (temperature factor,  $P \leq 0.018$ ). In addition, germination responses across the temperature range varied among populations for *Physalis* and *Asclepias* (population factor and population x temperature,  $P \leq 0.0001$ ). Fresh seeds of both *Penstemon* species germinated up to 9% with highest germination at 30/15°C; no germination occurred at temperatures  $\leq 20/10$  or 25/15°C depending on the species (Figure 1). Highest germination for both populations of fresh *Physalis* seeds was at 30/15°C, with decreasing amounts at reduced temperatures; no germination occurred at  $\leq 15/6$ °C. For *Asclepias*, fresh seeds from IL and MO populations germinated highest at 30/15°C but those from MN germinated highest at 20/10°C. No germination occurred at  $\leq 15/6$  or 20/10°C of seeds from all three *Asclepias* populations.

Control seeds germinated significantly different among temperatures for the four species (temperature factor,  $P \leq 0.0001$ ). Although germination responses across the temperature range varied among populations for *Asclepias* (population factor and population x temperature,  $P \leq 0.0001$ ), they did not do so between populations of *Physalis* ( $P \geq 0.204$ ). Germination of control seeds for *Penstemon* increased over time,

particularly at 15/6 and 5°C depending on the species (Figure 2). In contrast, control seeds of *Physalis* increased most dramatically over time at 20/10, 25/15 and/or 30/15°C. Germination of control seeds from *Asclepias* increased mostly at 15/6 and 20/10°C for populations in IL and MN and at 15/6, 20/10, and 30/15°C in MO.

**Germination of stratified seeds:** For both species of *Penstemon*, germination varied significantly over time and with stratification temperature (factors and interaction,  $P \leq 0.0001$ ). With increased duration of stratification at 1°C, germination increased up to 62-87% (Figures 3, 4). In contrast, the highest germination observed during stratification at 5°C was 49% for *P. digitalis* and 34% for *P. tubaefflorus*. Germination for both species following stratification at 9°C was mostly  $\leq 12\%$ .

Both populations of *Physalis* responded to the three stratification temperatures over time in the same manner (3-way interaction,  $P = 0.977$ ). Germination at 15/6 and 30/15°C remained  $\leq 32\%$  and  $\geq 70\%$ , respectively, regardless of the length of stratification at 1, 5, or 9°C for both populations (Figures 5, 6). Compared to fresh seeds, germination at 20/10 and 25/15°C increased particularly following 4 and/or 8 weeks of stratification at 1, 5, and 9°C for the WI population and at 5 and 9°C for the MN population. For the MN population stratified at 1°C, germination at 20/10 and 25/15°C gradually increased over the 4 to 20 weeks of stratification.

The three populations of *Asclepias* showed similar responses to stratification length, stratification temperature, and incubation temperature (4-way interaction,  $P = 0.077$ ). As compared to fresh seeds, germination rapidly increased and then remained relatively constant ( $\geq 73\%$ ) at 20/10, 25/15, and 30/15°C with 4-20 weeks of stratification

at 1, 5, and 9°C (Figures 7-9). In contrast, germination slowly increased at 15/6°C with increasing amounts of stratification at 1, 5, and 9°C. However, the highest germination at 15/6°C varied between 40-77%, depending on duration of stratification and stratification temperature.

**Temperature tolerance ranges:** The TRs for fresh and cold-stratified seeds did not differ between populations of *Physalis*, but they did so among populations of *Asclepias* ( $P < 0.0001$ ). TRs for fresh seeds for gravity-dispersed *Penstemon* was significantly less than those for the animal-dispersed *Physalis* and wind-dispersed *Asclepias*, with the TRs of the latter two dispersal strategies not differing ( $P < 0.0001$ ) (Figure 10). For cold-stratified seeds, TRs differed significantly among the three dispersal strategies ( $P = 0.001$ ) with the hierarchy (narrowest to widest) being: gravity < animal < wind (Figure 11).

**Sequence of temperature regimes:** Germination through the sequences differed significantly over time between current vs. simulated future conditions for both *Penstemon* species and *Physalis* (time, condition, time x condition,  $P \leq 0.033$ ). However, germination was similar over time for the two conditions for *Asclepias* (time, time x condition,  $P \geq 0.066$ ) with future germination being significantly higher than current germination (condition,  $P < 0.0001$ ). Although both populations of *Physalis* responded similarly over time to both conditions (population x condition,  $P = 0.427$ ), germination for both was higher under simulated future than current conditions ( $P = 0.001$ ) and was higher for the WI than for the MO population ( $P < 0.0001$ ).

Germination for both *Penstemon* occurred at the same time (late winter/spring months) for both simulated future and current conditions, with *P. digitalis* germinating higher under future conditions and *P. tubaefflorus* under current conditions (Figure 12). In contrast, germination shifted to autumn months under simulated future conditions as compared to late winter/spring months under current conditions for *Asclepias*.

Germination for both *Physalis* populations occurred in autumn months under current and simulated future conditions, with little additional germination occurring during winter or spring months.



Table 3. Seed mass of the study species and populations.

Species	State	Location	Mean $\pm$ SE (g)
<i>Penstemon digitalis</i>	IL	Nachusa Grasslands	0.0072 $\pm$ 0.0001
<i>Penstemon tubaeflorus</i>	MO	Tingler Prairie Conservation Area	0.0049 $\pm$ 0.0020
<i>Physalis longifolia</i> var. <i>subglabrata</i>	MN	MN Landscape Arboretum	0.0184 $\pm$ 0.0003
	WI	Lone Rock Prairie	0.0170 $\pm$ 0.0004
<i>Asclepias syriaca</i>	MO	Along west Outer Road just west of the intersection of I-44 and MO Hwy 141	0.1646 $\pm$ 0.0030
	IL	Nachusa Grasslands	0.1437 $\pm$ 0.0029
	MN	MN Landscape Arboretum	0.1288 $\pm$ 0.0024

\* Seeds collected in autumn 2014 from the same population as autumn 2013 were used to determine seed mass.

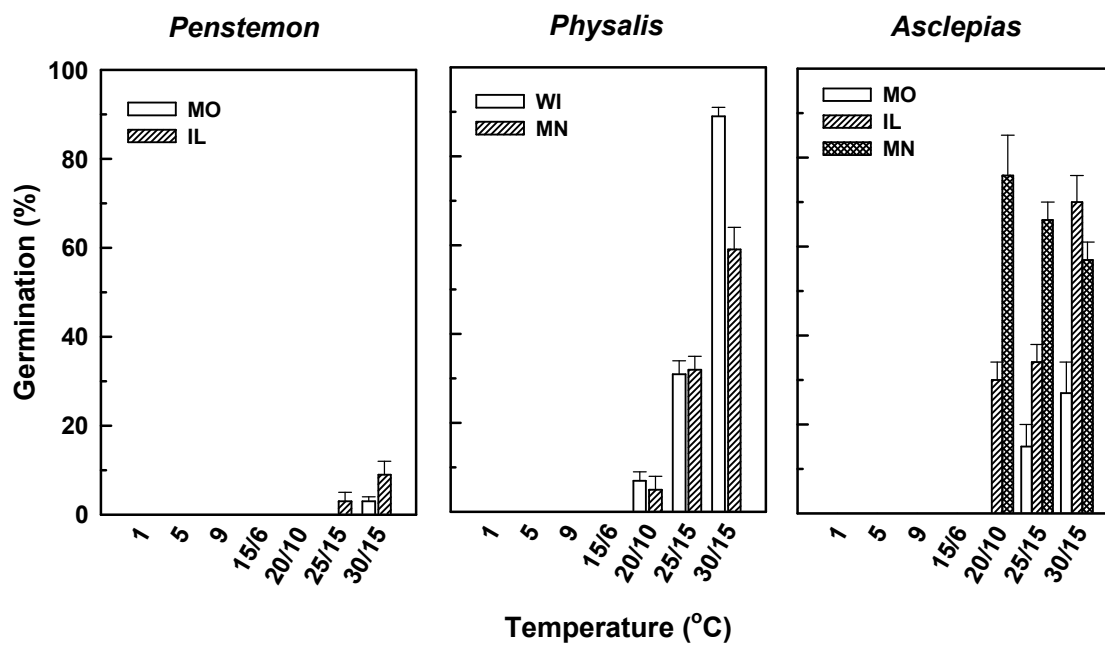


Figure 1. Germination (mean  $\pm$  SE) across temperatures for fresh seeds of *Penstemon digitalis* (IL), *P. tubaeiflorus* (MO), *Physalis longifolia* var. *subglabrata* (WI, MN), and *Asclepias syriaca* (MO, IL, MN). Seeds were incubated in light for 2 weeks.

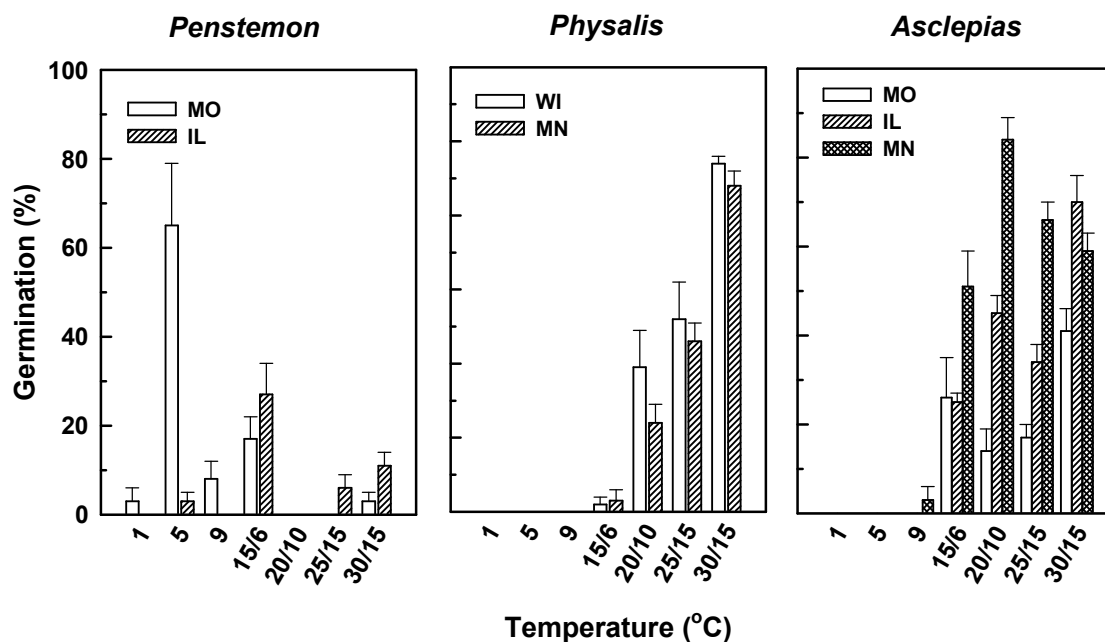


Figure 2. Germination (mean  $\pm$  SE) across temperatures for control seeds of *Penstemon digitalis* (IL), *P. tubaeiflorus* (MO), *Physalis longifolia* var. *subglabrata* (WI, MN), and *Asclepias syriaca* (MO, IL, MN). Seeds were incubated in light for 22 weeks.

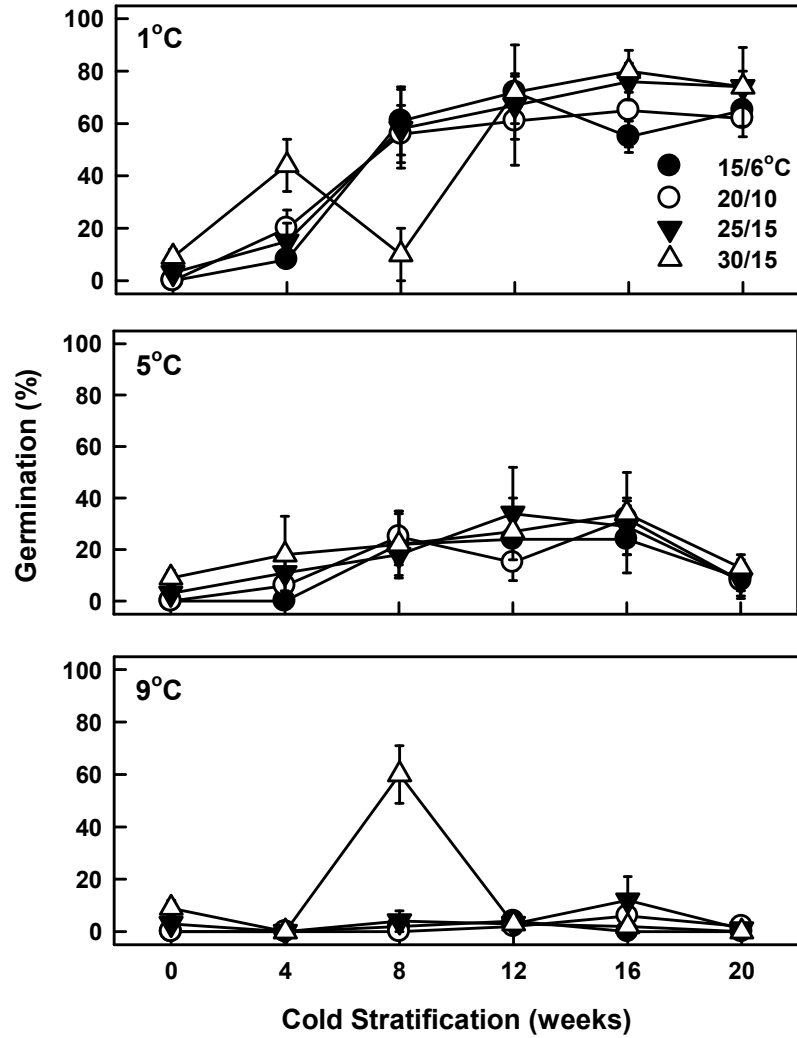


Figure 3. Germination (mean  $\pm$  SE) for stratified seeds of *Penstemon digitalis* from IL. Seeds were stratified at 1, 5, and 9°C in light for 0 (fresh), 4, 8, 12, 16, and 20 weeks and then incubated across the range of alternating temperatures in light.

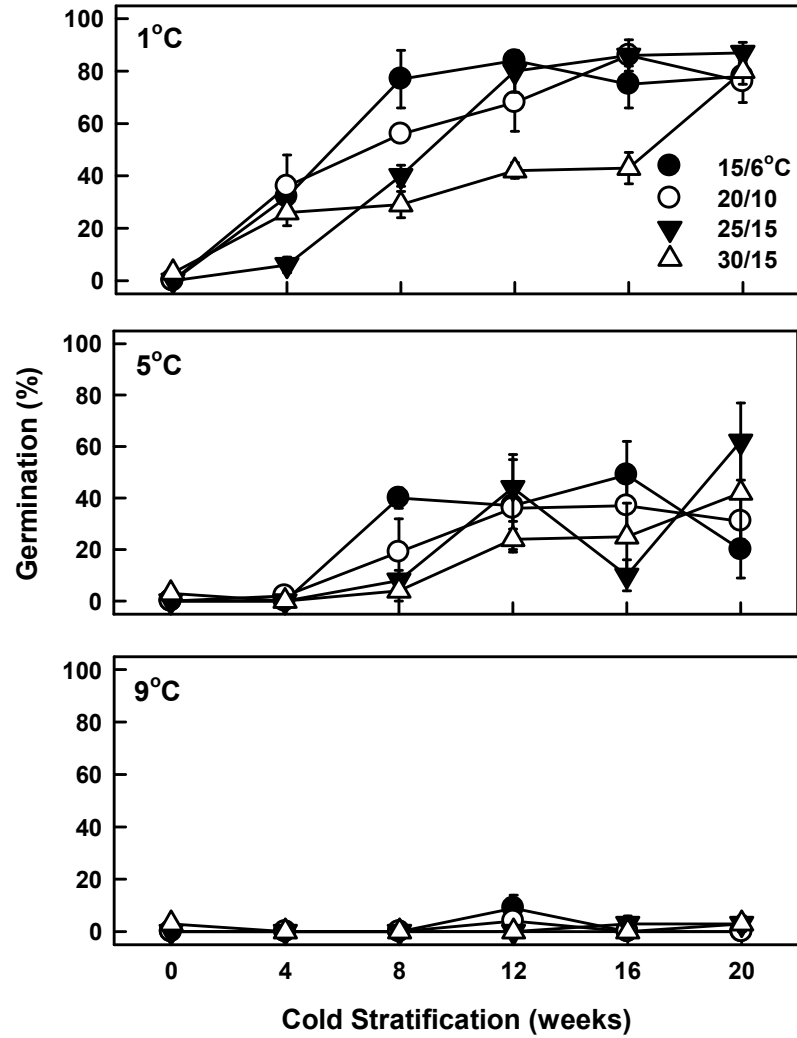


Figure 4. Germination (mean  $\pm$  SE) for stratified seeds of *Penstemon tubaeiflorus* from MO. Seeds were stratified at 1, 5, and 9°C in light for 0 (fresh), 4, 8, 12, 16, and 20 weeks and then incubated across the range of alternating temperatures in light.

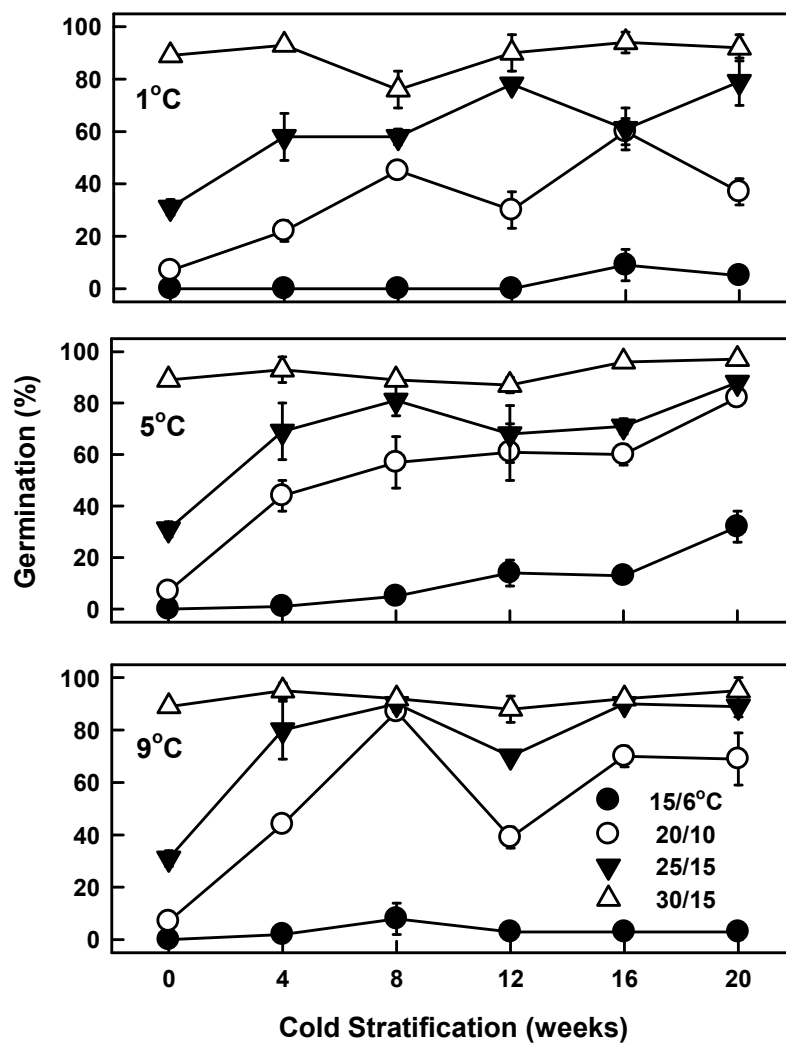


Figure 5. Germination (mean  $\pm$  SE) for stratified seeds of *Physalis longifolia* var. *subglabrata* from WI. Seeds were stratified at 1, 5, and 9°C in light for 0 (fresh), 4, 8, 12, 16, and 20 weeks and then incubated across the range of alternating temperatures in light.

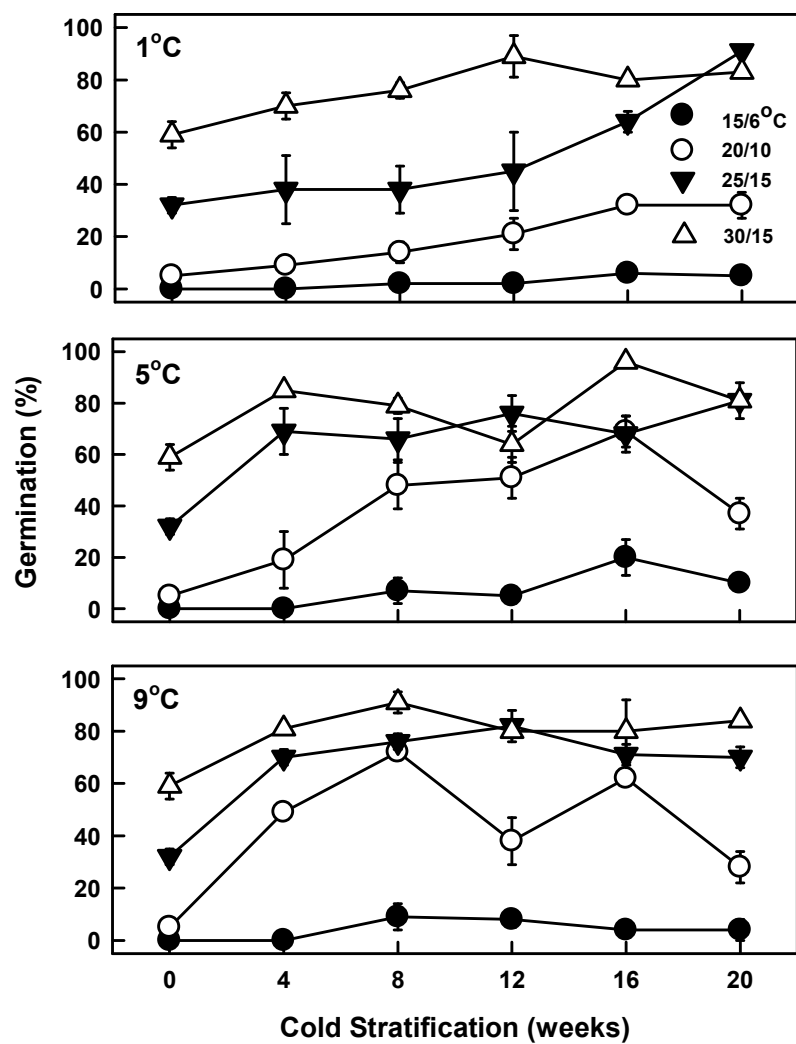


Figure 6. Germination (mean  $\pm$  SE) for stratified seeds of *Physalis longifolia* var. *subglabrata* from MN. Seeds were stratified at 1, 5, and 9°C in light for 0 (fresh), 4, 8, 12, 16, and 20 weeks and then incubated across the range of alternating temperatures in light.

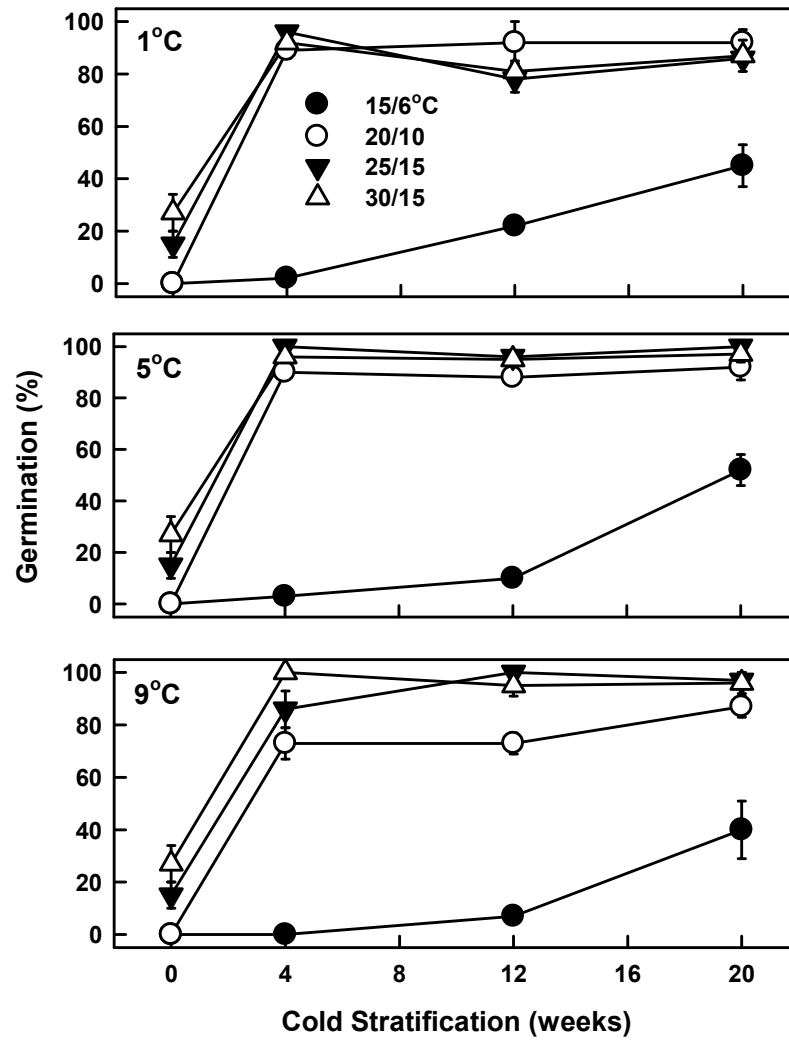


Figure 7. Germination (mean  $\pm$  SE) for stratified seeds of *Asclepias syriaca* from MO. Seeds were stratified at 1, 5, and 9°C in light for 0 (fresh), 4, 8, 12, 16, and 20 weeks and then incubated across the range of alternating temperatures in light.



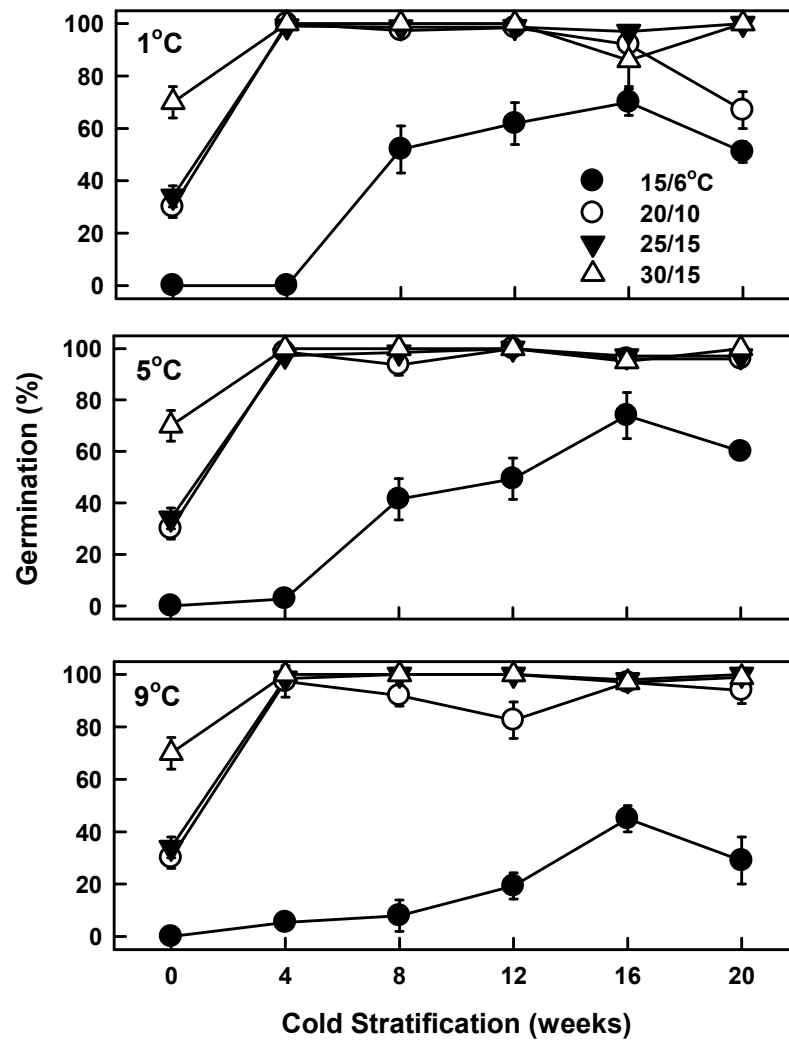


Figure 8. Germination (mean  $\pm$  SE) for stratified seeds of *Asclepias syriaca* from IL. Seeds were stratified at 1, 5, and 9°C in light for 0 (fresh), 4, 8, 12, 16, and 20 weeks and then incubated across the range of alternating temperatures in light.

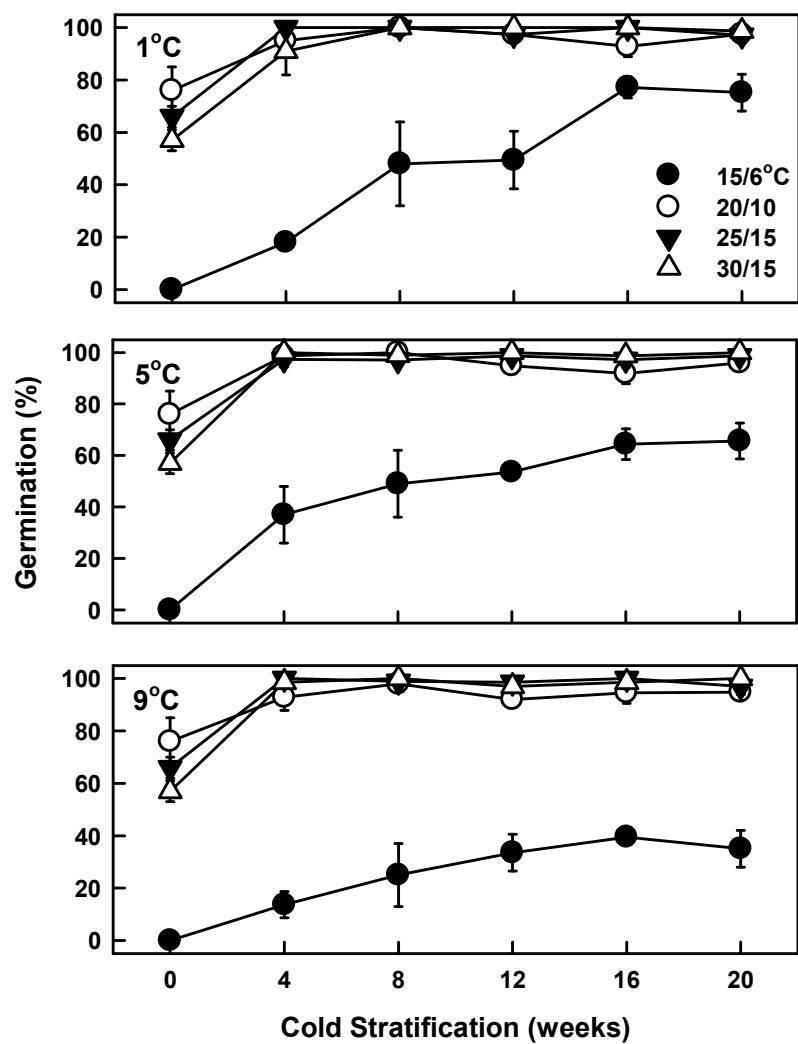


Figure 9. Germination (mean  $\pm$  SE) for stratified seeds of *Asclepias syriaca* from MN. Seeds were stratified at 1, 5, and 9°C in light for 0 (fresh), 4, 8, 12, 16, and 20 weeks and then incubated across the range of alternating temperatures in light.

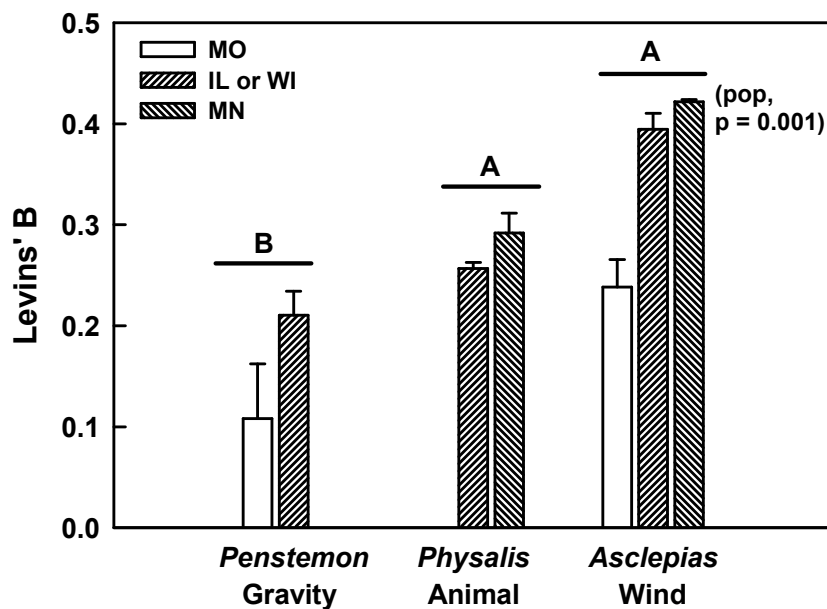


Figure 10. Temperature tolerance ranges (mean  $\pm$  SE), measured as Levins' B, for fresh seeds from three genera differing in dispersal strategies. Groups of bars with dissimilar letters are significantly different (PLSD,  $P \leq 0.05$ ); tolerance ranges differed among populations of *Asclepias* but not between populations of *Physalis*. The study species were: *Penstemon digitalis* (a population from IL), *Penstemon tubaeiflorus* (a population from MO), *Physalis longifolia* var. *subglabrata* (populations in WI, MN), and *Asclepias syriaca* (populations from MO, IL, MN).

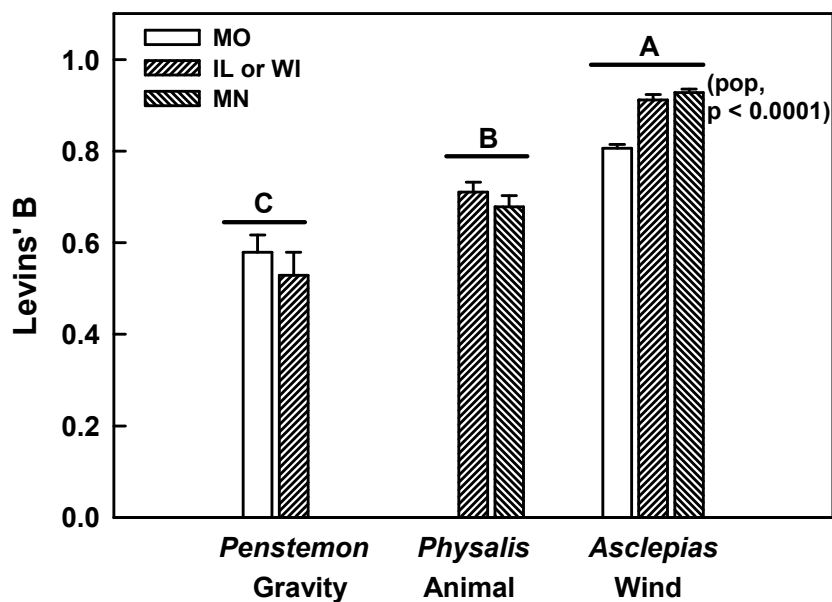


Figure 11. Temperature tolerance ranges (mean  $\pm$  SE), measured as Levins' B, for 12-week cold-stratified seeds from three genera differing in dispersal strategies. Groups of bars with dissimilar letters are significantly different (PLSD,  $P \leq 0.05$ ); tolerance ranges differed among populations of *Asclepias* but not between populations of *Physalis*. The study species were: *Penstemon digitalis* (a population from IL), *Penstemon tubaeiflorus* (a population from MO), *Physalis longifolia* var. *subglabrata* (populations in WI, MN), and *Asclepias syriaca* (populations from MO, IL, MN).

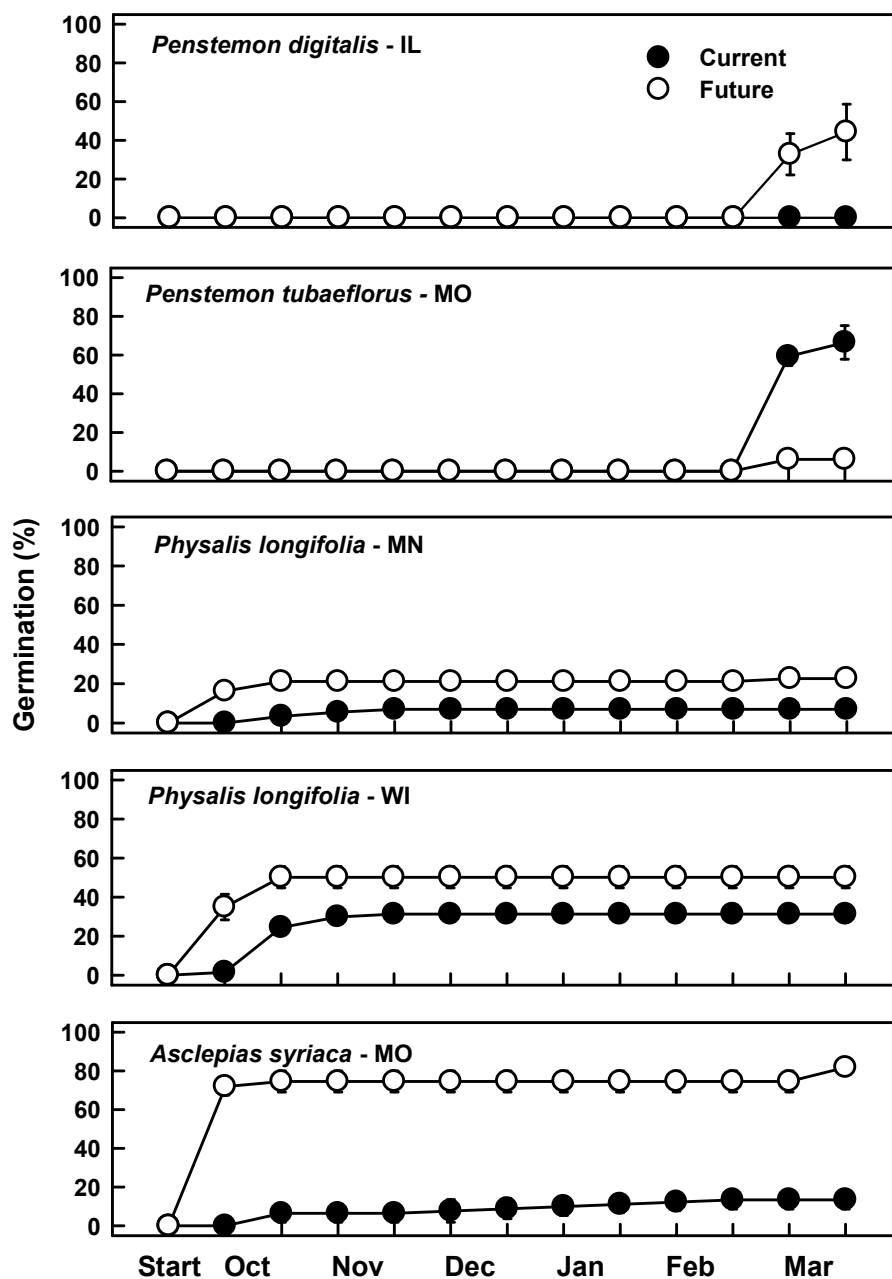


Figure 12. Germination (mean  $\pm$  SE) of seeds moved through a series of temperatures (see Table 2) simulating the time between dispersal and germination under current and future (+5°C) conditions.

## CHAPTER IV

### DISCUSSION

Fresh seeds of both *Penstemon* germinated to low percentages across the temperature range, whereas those of *Physalis* and *Asclepias* germinated to low percentages at  $<15/6^{\circ}\text{C}$  and from moderate to high percentages at  $\geq 25/15^{\circ}\text{C}$ , regardless of the population location (Figure 1). At  $20/10^{\circ}\text{C}$ , low germination occurred for *Physalis* from WI and MN and from moderate to high germination occurred for *Asclepias* from IL and MN but no germination from MO. Thus, seeds of *Penstemon* were dormant at maturity while those of *Physalis* and *Asclepias* were conditionally dormant (sensu Baskin and Baskin, 1998).

Seed dispersal of the study species occurs during autumn when habitat temperatures are  $15/6$  or  $20/10^{\circ}\text{C}$  (Table 2). At these temperatures, I would expect no germination during autumn for *Penstemon* and very low germination for both populations of *Physalis*. In populations of *Asclepias* from MO and MN, seeds are not capable of germinating at temperatures that occur during dispersal ( $20/10$  and  $15/6^{\circ}\text{C}$ , respectively). In contrast, moderate germination could occur in populations of *Asclepias* from IL if dispersal occurred in early autumn ( $20/10^{\circ}\text{C}$ ) but no germination in late autumn ( $15/6^{\circ}\text{C}$ ). This period of dormancy or conditional dormancy can be considered a mechanism to prevent germination in autumn, when seedlings would not have enough time to establish and grow before the freezing conditions of winter (Walck *et al.*, 1997).

During winter, cold stratification occurs that alleviates dormancy (Baskin and Baskin, 1998). I simulated this winter condition by keeping seeds moist at 1, 5,  $9^{\circ}\text{C}$  for

up to 20 weeks. In contrast to fresh seeds, cold-stratified seeds of *Penstemon* germinated to high percentages at 15/6-30/15°C (Figures 3, 4), *Physalis* germinated to high percentages at 20/10°C (Figures 5, 6), and *Asclepias* germinated from moderate to high percentages at 15/6-30/15°C (Figures 7-9). Specifically, the simulated cold winter condition enabled seeds of all four species to germinate at 15/6 and/or 20/10°C which they could not do so when fresh (i.e. soon after autumn dispersal). Thus, following cold stratification seeds of the study species would be able to germinate in nature in spring at temperatures (15/6 or 20/10°C) that prevented germination in autumn.

The optimum temperature for and duration of cold stratification to overcome dormancy differed among the four species and populations. Dormancy break was more effective at 1°C than at 5 or 9°C for the two *Penstemon* species (Figures 3, 4), whereas it was about equally effective at 1, 5, and 9°C for *Physalis* (Figures 5, 6) and *Asclepias* (Figures 7-9). Even though there were no significant differences in germination responses among the stratification temperatures of 1, 5, or 9°C for *Physalis* and *Asclepias*, the duration of stratification needed to achieve high germination differed between the species. Both *Physalis* populations required more than 8 weeks of cold stratification for high germination at  $\geq 20/10^\circ\text{C}$ ; germination remained low at 15/6°C regardless of stratification duration (Figures 5, 6). In contrast, *Asclepias* populations had high germination at temperatures  $\geq 20/10^\circ\text{C}$  following only 4 weeks of stratification; highest germination at 15/6°C occurred following 20 weeks of stratification (Figures 7-9). Baskin and Baskin (1977) reported that seeds of *Asclepias syriaca* germinated to high percentages at 30/15 and 35/20°C following 2 weeks of stratification at 5°C and at 20/10°C following 9 weeks of stratification; seeds never germinated at 15/6°C regardless

of the length of stratification. In contrast to Baskin and Baskin (1977), seeds of *Asclepias* in my study germinated at 15/6°C following cold stratification.

Fresh seeds of both *Physalis* and *Asclepias* had highest germination at 30/15°C and lowest at 15/6°C (Figures 5-9). However, the responses of seeds of *Physalis* and *Asclepias* to increasing durations of cold stratification differed. Germination at 15/6°C gradually increased with increased cold stratification in *Asclepias*, reaching the highest (70%) percentage after 16 or 20 weeks of cold stratification (Figures 7-9). This gradual increase in germination was not observed in *Physalis* populations (Figures 5, 6). Instead, germination at 15/6°C remained low even after 20 weeks of stratification. Based upon the temperature difference at 15/6°C between *Physalis* and *Asclepias*, I suggest that in the field seedling emergence would occur earlier for *Asclepias* as compared to *Physalis*.

The tolerance ranges for fresh seeds were relatively narrow (< 0.5) and this narrow range is partly explained by the seeds being dormant or conditionally dormant (Figure 10). That is, as dormancy is alleviated, the temperature range over which germination occurs broadens (Baskin and Baskin 1998). Moreover, the tolerance range for the fresh seeds of gravity dispersed *Penstemon* species were significantly narrower than that of the *Asclepias* and *Physalis* (Figure 10). Fresh seeds of *Penstemon* did not germinate in autumn (Figure 1) because the autumn temperatures are beyond the narrow tolerance range, i.e. their seeds remain in the dormant stage after dispersal. In contrast, as the autumn temperatures are within the tolerance range of the conditionally dormant *Asclepias* and *Physalis* fresh seeds (Figure 1), they are capable of germinating to low percentages after dispersal.



Cold stratification, which serves as a dormancy break mechanism, widens the germination temperature range of a species (Baskin and Baskin, 1998). The results of this study showed that the 12 weeks of cold stratification widened the tolerance range of all the study species, enabling the non-dormant seeds to germinate over a broad range of temperatures. Hence, the values of Levins' B for non-dormant seeds were higher than those of fresh seeds (Figures 10, 11). The level of tolerance range increase after cold stratification was species dependent: *Asclepias* populations widened the tolerance range to a maximum level (near 1), *Physalis* had a relatively medium increase and *Penstemon* species increased the least. This results suggest that the temperature tolerance ranges are mediated by the species specific life history characters.

In the current study, I hypothesized that the tolerance range differences among species could be explained by the seed dispersal strategies. As I predicted, my results showed that there was a correlation between the temperature tolerance range for germination and the seed dispersal strategy. The *Asclepias* populations (wind dispersed) had the broadest tolerance range and *Penstemon* (gravity dispersed) had the narrowest tolerance range; *Physalis* (animal dispersed) had a medium tolerance range. The tolerance range is a representation of the germination niche of a species (Cochrane *et al.*, 2011), and thus, the current results can be used to interpret the niche differences with respect to the dispersal events. Anguilée *et al.* (2012) suggested that species with seed dispersal mechanisms have the potential to acquire a wide niche breadth through the process of niche expansion. Therefore, it is possible that wind dispersed species such as *Asclepias*, have a broad niche compared to the gravity dispersed *Penstemon*. Thus, *Asclepias*, may have the potential to withstand a wide range of temperature conditions, colonize new

habitats and persist in natural habitats under changing environmental conditions. One drawback of the Levins' B value is that, it represents only the width of the tolerance range and it does not give the exact temperature values of the temperature range ( $T_{opt}$ ,  $T_{min}$  and  $T_{max}$ ). Nevertheless, with the present results, it is possible to suggest that seed dispersal is one of the life history characters that determines germination temperature tolerance range and, the species with dispersal mechanisms (animal and wind) have wider tolerance range than the gravity dispersed species.

There was no significant difference in the tolerance ranges of non-dormant seeds among the populations of *Physalis* (Figure 11). In contrast, the southern population (MO) of *Asclepias* had a significantly narrower tolerance range than northern populations (IL and MN). This difference is somewhat in accordance with the seasonality hypothesis, which states that the populations at northern latitudes and high altitudes have broader tolerance ranges, as they experience greater annual temperature variation than southern and lower altitude populations (De Frenne *et al.*, 2012; Orru *et al.*, 2012). However, it is important to carry out future studies with more populations across a latitudinal gradient in order to see the above pattern in the study species persists. Although Farmer *et al.* (1986) did not investigate tolerance ranges, they reported that the germination of *Asclepias syriaca* differed among populations that they sampled throughout eastern United States.

Furthermore, the tolerance range results can be used to predict the responses of the study species to a warming climate. Species with narrow germination niche or tolerance range (Cochrane *et al.*, 2011) and limited dispersal ability (Engler *et al.*, 2009) have been reported to be highly threatened by climate change (Walck *et al.*, 2011). Therefore, the gravity dispersed *Penstemon* species has the highest risk of impact with

climate change. Some species can withstand such threats through phenological shifts (Walck *et al.*, 2011), but our move along experiments (Figure 12) showed that both *Penstemon* species did not shift their germination timing under simulated future climatic conditions. Thus, persistence of the study populations of the two *Penstemon* species in their current location may not be possible if the temperature increase is outside the tolerance range. These populations would need to rely on natural selection and/or on migration. In contrast, populations of *Physalis* and *Asclepias* could persist in their current locations due to their wide tolerance range.

Changes in germination phenology were highly species specific (Figure 12). While *P. digitalis* had increased germination under simulated future temperature conditions, *P. tubaeiflorus* had reduced germination. Both *Physalis* populations had increased germination under future conditions and germination shifted slightly earlier. In contrast, *Asclepias* shifted its germination to autumn under future conditions. However, it is questionable whether these seedlings that emerge in autumn would survive over winter. Therefore even though the species with the broadest tolerance range can germinate to high percentages at a different time in the future, it is difficult to predict whether these new seedlings will support population persistence in the future.

The combined results of tolerance range (Figures 10, 11) and move along studies (Figure 12) can be used to interpret future responses of the study species under climate change. Most studies that predict future responses and distributions of plants with climate change have neglected the effect of seed dispersal and make predictions under unlimited or null seed dispersion (Thomas *et al.*, 2004). In contrast, other studies have emphasized the importance of dispersal scenarios in climatic models (Engler *et al.*, 2009; Bateman *et*

*al.*, 2013). Our results support the second idea. The dispersal strategy of a species is an important life history character for predicting species' responses under climate change. Herein, I have shown how dispersal strategy plays an important role in determining the germination tolerance range of a particular species. Given the germination responses for *Asclepias* and *Physalis*, populations of these species could persist in their current distribution range since their tolerance ranges are broad enough to encompass a wide range of predicted future temperatures. In contrast, the persistence of *Penstemon* populations within their distribution range is more limited given the narrower tolerance range, especially for dormancy break, compared to *Asclepias* and *Physalis*.

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