Investigating germination of *Asclepias syriaca* in response to climate change

Courtney Devoid[1], Jessamine Finch[2,3]

1. Middlebury College, Middlebury, Vt. 2. Chicago Botanic Garden, Glencoe, IL; 3. Northwestern University, Evanston, IL.

Introduction

Global climate change is projected to cause shifts in multiple climatic variables throughout the next century. This is predicted to impact critical stages in plant reproduction, which are dependent on specific environmental conditions. Seed dormancy break, germination, and seedling establishment are important stages in plant regeneration that have specific and optimum climate conditions (Walck et al. 2011). These conditions are bounded by maximum and minimum thresholds, resulting in locally adapted environmental tolerance ranges. A rapid change in climate could result in a disconnect between the conditions experienced and the conditions required for germination, which could result in decreased population fitness and reproduction rates (Cochrane 2011). In order to examine the potential impacts of climate change on locally adapted populations, this project looked at populations located in three states along a latitudinal gradient. The states were from unique hardiness zones, meaning they experience documented annual differences in climate (USDA 2012). The seeds were refrigerated to simulate different winter lengths and germinated across a range of incubation temperatures. These treatments allowed us to examine local adaptation and the impacts of climate on germination rates.

Objectives:

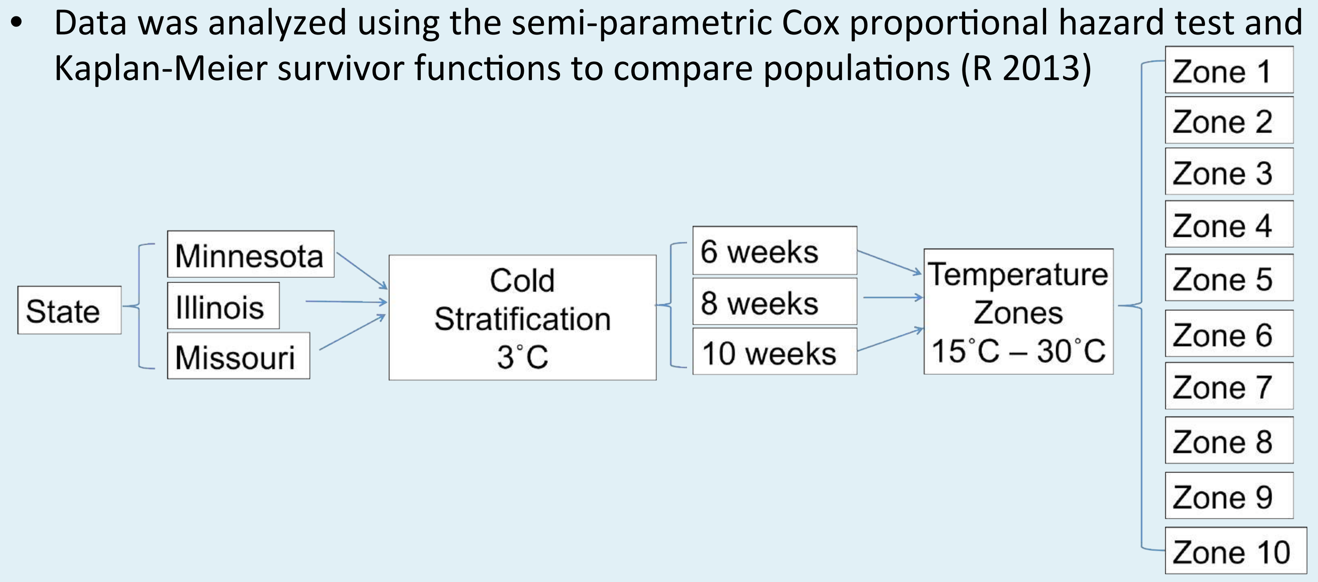
- Determine if germination rates are locally adapted to a hardiness zones climatic conditions.
- Determine if stratification length impacts germination rate for *Asclepias syriaca*.
- Determine if colder temperature zones will experience lower germination rates than higher temperature zones.

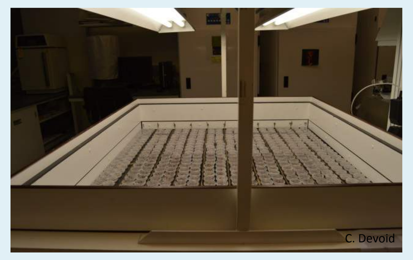
Hypothesis:

Populations of *Asclepias syriaca* will have distinct temperature and cold stratification tolerance ranges dependent on latitudinal seed source.

Methods:

- Seeds were bulk collected from three populations located along a latitudinal gradient (Minneapolis, MN; Chicago, IL; and St. Louis, MO)
- Each population was subject to three cold stratification treatments of six weeks, eight weeks, and ten weeks at 3°C
- Germination was tested using a thermogradient table with ten temperature zones ranging from 15 °C to 30 °C
- Six replicates were used for each treatment and population. Each replicate contained eight seeds placed in 35 mm petri dishes on a 1.5% agar solution
- Two rows of dishes were placed in each zone, rows were switched each day so plates experience the same temperatures on average. Data was collected every other day
- Seed viability was assessed through a random sample of 100 seeds per population using x-ray analysis (Specimen Radiography System, Faxitron X-Ray Corporation) and viability adjusted germination was calculated
- Data was analyzed using the semi-parametric Cox proportional hazard test and Kaplan-Meier survivor functions to compare populations (R 2013)





Study Species: *Asclepias syriaca*

Perennial with creeping rootstocks, reproduces by seed and root buds, simple stems, contains milky latex in all parts of plant, requires cold stratification to break seed dormancy, requires 18-32˚C mean July temperatures and minimum of 50 cm rainfall. *Asclepias syriaca* L. (Apocynaceae) is an important species for monarch butterfly conservation (Bhowmik and Bandeen 1976, Baskin and Baskin 1997).

Study Sites:

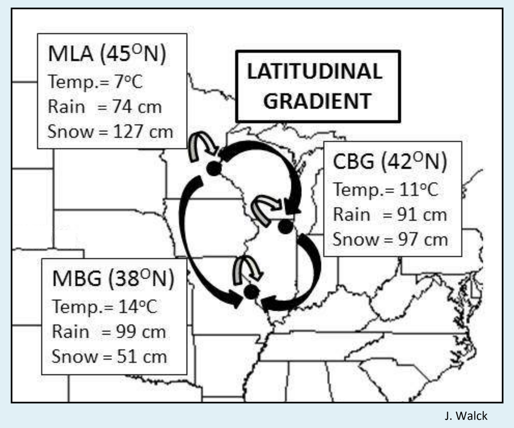


Figure 1: A map depicting the locations seeds were collected along a latitudinal gradient at Minnesota Landscape Arboretum (MLA), Chicago Botanic Garden (CBG), and Missouri Botanic Garden (MBG). Locations are all in different hardiness zone, as designated by the USDA (2012).

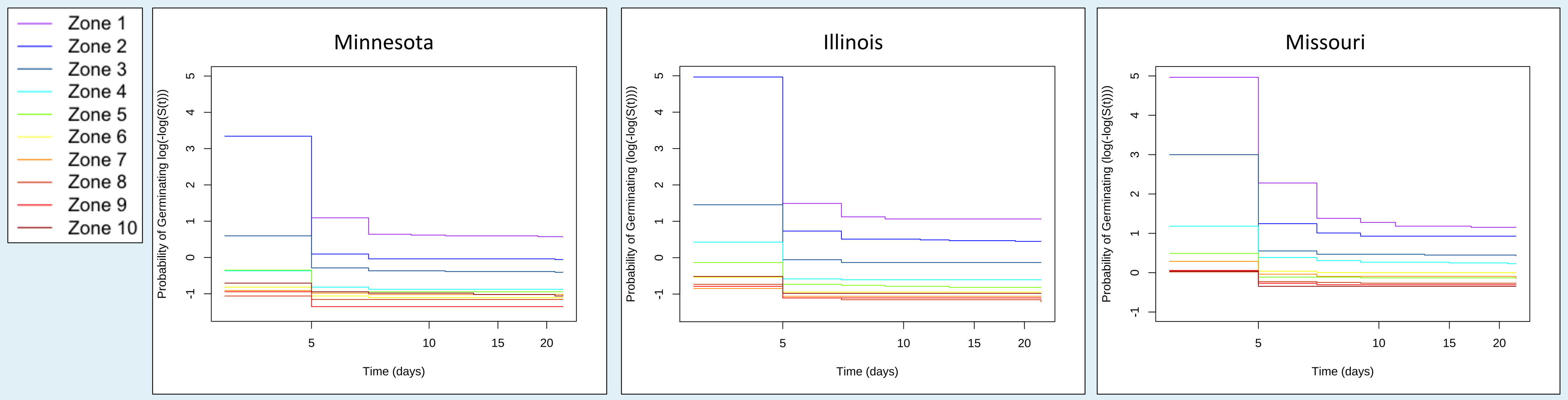


Figure 2: Kaplan- Meier survivorship curves showing the probability of germination by zone over time. Colder zones experienced a higher probability of germination than warmer zones, meaning they had lower germination rates. In general, colder zones had curves with larger spread out warmer zones. For all states, zone 1 had the highest probability of germination by the end of the experiment, and the lowest germination count. Zones 8-10 had the lowest probability of germination and highest germination count.

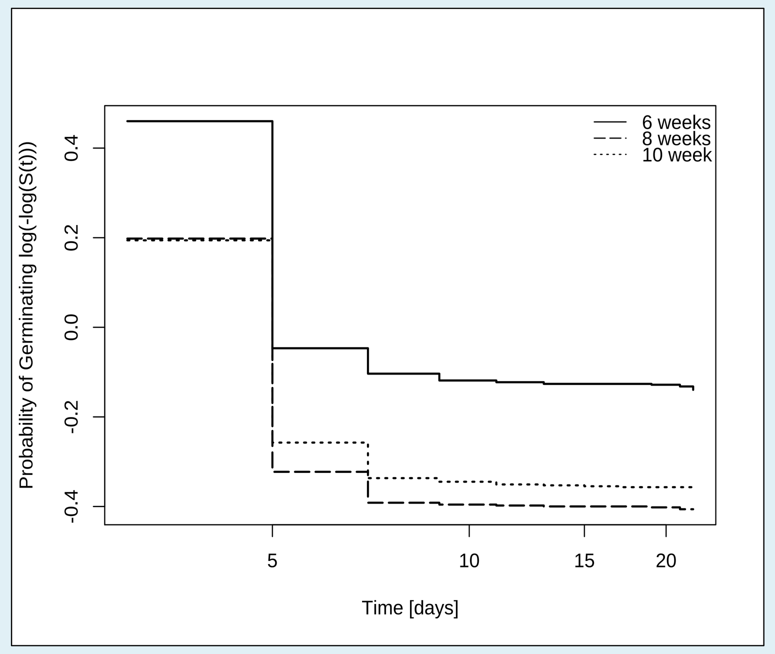


Figure 3: Kaplan- Meier survivor functions showing the probability of germination over time for cold stratification periods. The six week period had the highest probability of germination and the eight week period had the lowest probability of germination .

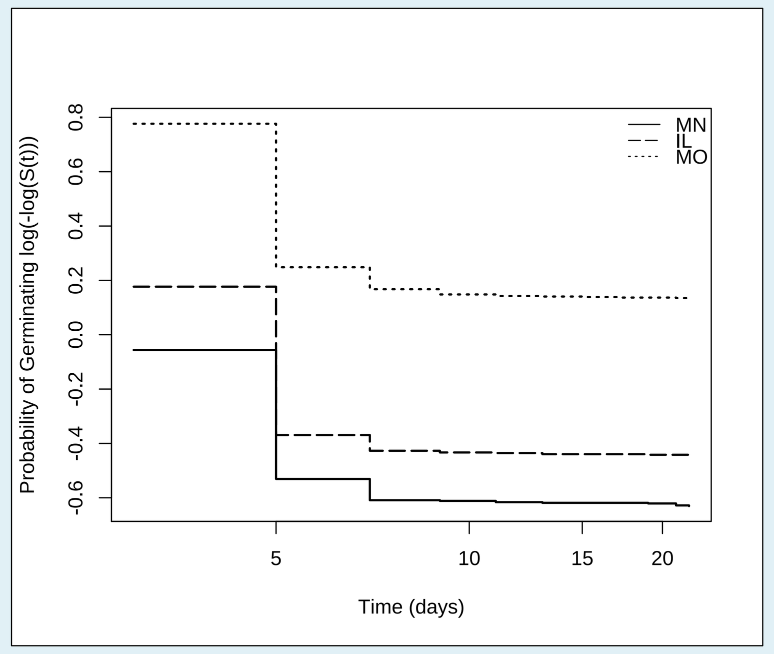


Figure 4: Kaplan- Meier survivor functions showing the probability of germination over time by state. Missouri had the highest probability of germination and Minnesota had the lowest probability.

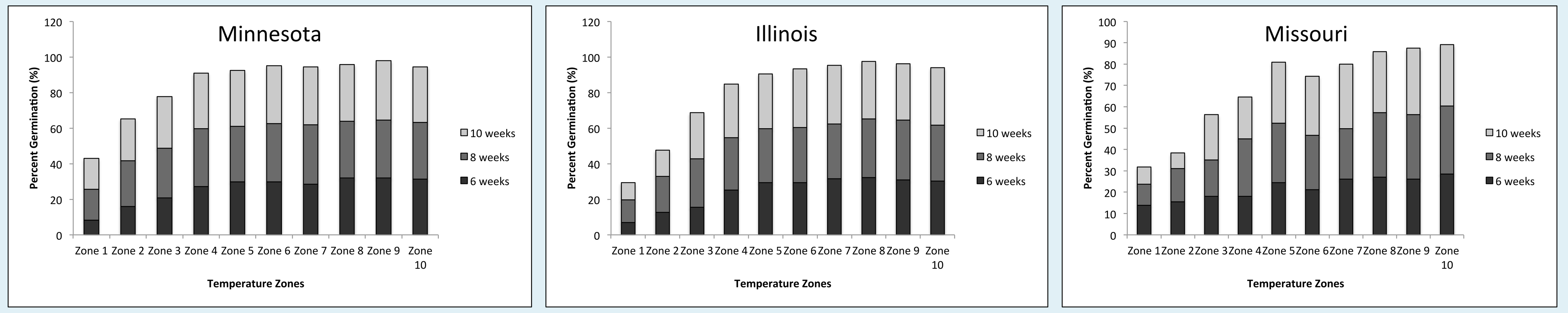


Figure 5: Percent germination by state divided into cold stratification periods. The greatest difference between states is observed in the colder zones. Minnesota had the highest germination for the 10 week cold stratification period in colder zones, compared to the other states. Missouri had the highest germination for the 6 week stratification period in the colder zones.

Results:

- State, temperature zone, and cold stratification period all had significant effects on germination rates (Cox proportional hazard test, $R^2 = 0.283$; state: $p = 0.0273$, zone: $p < 0.001$, stratification length: $p < 0.001$).
- Stratification length and state were significant interaction variables ($p = 0.0443$)
- The eight and ten week cold stratification periods had lower probability of germination than the six week cold stratification period for all three states.
- Minnesota and Illinois had a lower probability of germination than Missouri, meaning that these states had greater total germination.

Table 1: Summary table of the best fit Cox model as produced by the R function coxph (). $h_i(t)$= Survival (Day, Status) ~ Strat. Length + State + Zone + Strat. Length : State). SE denotes standard error.

Covariate	Coefficient	Exp	SE	z	p value
Strat. Length	0.1203	1.1279	0.0265	4.537	5.7e-06 ***
State	-0.2342	0.7918	0.1061	-2.207	0.0273 *
Zone	0.2012	1.223	0.006	33.368	< 2e-16 ***
Strat. Length:Zone	-0.026	0.9743	0.0123	-2.012	0.0443 *

Signif. codes: '***' 0.001 '**' 0.01 '*' 0.05.

Concordance= 0.808 (se = 0.011)
R^2= 0.283 (max possible= 1)
Likelihood ratio test= 1439 on 4 df, p=0
Wald test = 1450 on 4 df, p=0
Score (logrank) test = 1515 on 4 df, p=0

Conclusion:

Germination responses of *A. syriaca* are impacted by the state of origin, length of cold stratification, and incubation temperature. This suggests that local populations experiencing different annual climates are adapted to the region of origin. It is predicted that climate change will cause an increase in temperatures, resulting in earlier spring temperatures and shortened stratification length (IPCC 2007). The Kaplan-Meier curves showed that shorter stratification lengths had a higher probability of germination, hence less actual germination was observed. *Asclepias syriaca* requires cold stratification for dormancy break, and if this condition is not met because the winters are too short, po pulation recruitment will be effected. The Kaplan-Meier curves for temperature zones showed that colder zones experienced less germination. This result, along with forecasted increases in spring temperatures could mean in an initial increase in germination rates. This presents a trade off between increase in germination with rising spring temperatures and decreased winter length due to shortened stratification period. With rapid change in climate, populations may not be able to adjust their distribution quick enough, causing further decline (Frenne et al. 2011).

Future Work:

The temperature zones tested did not reach the upper temperature threshold for *A. syriaca*. Future work should include shifting the range of temperatures tested until the maximum threshold is reached. This information will be especially important for predicting the impact of climate change on *A. syriaca* in southern parts of the country. Future work should also include looking at maternal genetic origins for intra-specific population differences at each site. This would inform the range of differences that occur locally within a population. Examining the upper threshold of cold stratification would also be informative, as extreme weather events are predicted with climate change (IPCC 2007). This experiment looked at the required cold stratification period, but it is also possible that there could be to much cold stratification, creating the upper limit to the range of *A. syriaca*.

Acknowledgements:

We would like to thank NSF-REU grant 0648972 for support, along with the REU program mentors. We would also like to thank Kayri Havens-Young PhD for advising the project and College First intern Rachael So.

References:

1. Baskin, C.C., and J.M. Baskin. 2001. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press, Elsevier.
2. Bhowmik, P.C., and J.D. Bandeen. 1976. The biology of Canadian weeds 19. *Asclepias syriaca* L. Canadian Journal of Plant Science 56: 579-589.
3. Cochrane, A., M.I. Daws, and F.R. Hay. 2011. Seed-based approach for identifying flora at risk from climate warming. Australia Ecology 36 : 923-935.
4. Frenne, D.P., Frenne, D.P., J. Brunet, A. Shevtsova, A. Kolobs, B. J. Graae, O. Chabrerie, S.A. Cousins, G. Decocq, A. D. Schrijver, M. Diekmann, R. Grywez, T. Heinken, M. Hermy, C. Nilsson, S. Stanton, W. Tack, J. Willaert, and K. Verhenyen 2011. Temperature effects on forest herbs assessed by warming and transplant experiments along a latitudinal gradient.
5. IPCC, 2007: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and H.L. Miller (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
6. McNair, J., A. Sunkara, D. Forbish. 2013. How to analyze seed germination data using statistical time to event analysis: non=parametric and semi-parametric methods. Seed Science Research 22: 77-95.
7. Therneau , T. 2014. *A Package for Survival Analysis in S*. R package version 2.37-7, http://CRAN.R-project.org/package=survival.
8. Urbanek, S., H.J. Bibiko, S.M. Iacus. 2013. R 3.0.0 for Mac. The R Foundation for Statistical Computing.
9. USDA. 2012. USDA Plant Hardiness Zones Map. Accessed at: http://planthardiness.ars.usda.gov/PHZMWeb/
10. Walck, J.L., S.N. Hidayati, K.W. Dixon, K.Thompson, and P. Poschlod. 2011. Climate change and plant regeneration from seed. Global Change Biology 17: 2145-2161.