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MICHAEL KISTENMACHER
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BET-HEDGING IN HETEROCARPIC *GRINDELIA CILIATA* (ASTERACEAE)

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF MICROBIOLOGY AND PLANT BIOLOGY

BY

_____
Dr. J. Phil Gibson, Chair

_____
Dr. Bruce Hoagland

_____
Dr. Heather McCarthy

_____
Dr. Abigail Moore

_____
Dr. Lara Souza

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Abstract

A challenge for all living organisms is to offset the fitness associated risks of environmental uncertainty. Bet-hedging strategies are adaptive in unpredictable environments and are documented in a wide range of taxa spanning all kingdoms (Simons 2011, see Appendix A). There are two general types of bet-hedging strategies, a *conservative strategy* exemplified by the phrase "a jack of all trades and master of none" and the more common *diversified strategy* best described by the phrase "don't put all your eggs in one basket" (Seger and Brockman, 1987; Simons, 2011, see Appendix A). Although there are differences between them, both are based on the principle that mean geometric fitness increases by reducing fitness variation among years (Cohen, 1966; Gillespie, 1974; Philippi and Seger, 1989, see Appendix A).

In annual plants, small germination fractions and high seed survival in the seed bank are adaptive when good years occur unpredictably or in a low frequency. The opposite is true for environments when the probability of a good year is high. Mathematical models have derived these theoretical predictions (Cohen 1966; Levin *et al.* 1984; Venable and Brown, 1988; Venable and Lawlor 1980, see Appendix A), but empirical tests of these models remains problematic, primarily because documentation of changes in geometric mean fitness are not realized until different environmental conditions are experienced by the study organism. Since bet-hedging strategies are adapted on an evolutionary time scale, environmental conditions to hedge against may not be experienced in a human lifetime, or longer. Thus, field experiments are not optimal for testing bet-hedging theory. However a few long term observational studies support theoretical expectations (see Pake and Venable 1996; Venable, 2007, see

Appendix A), and long-term manipulative experiments that alter environmental conditions (e.g. precipitation frequency) are feasible approaches (Petru and Tielboerger 2008, see Appendix A). Modeling is another method for testing theoretical predictions, specifically by combining demographic models that are coupled with environmental models that alter soil temperature and soil water content. Demographic models have been applied to estimate population growth rates in desert annuals by deriving parameters such as reproductive output, recruitment from the seed bank, and survivorship in response to simulated precipitation regimes (Gremer and Venable 2014; Salguero-Gomez *et al.* 2012, see Appendix A).

Bet-hedging literature consists of a mixture of results that support and reject theoretical predictions, particularly in regard to seed germination and dormancy. This is due, in part, to the inability to incorporate mechanisms such as phenotypic plasticity and maternal effects that confound observations of demographic traits (i.e., seed production and seed dormancy) in natural populations. Another shortcoming is that comparisons of demographic traits at the population level are not incorporated into predictive models, resulting in hampered understanding of underlying genetic differences in the expression of bet hedging strategies, which are important variables determining population persistence and range dynamics. Lastly, and potentially the greatest shortcoming, is that risk avoidance from alternative selection agents (i.e., herbivores) are almost entirely non-existent. For example, frequent pre and post dispersal seed predation (i.e., granivory) may favor adaptations that reduce the risk of predation, such as increased lignification, or accumulation of tannins and similar defense compounds in fruit or seed coats (i.e., pericarp, and testa), that simultaneously alter germination or dormancy.

Since plant-animal interactions are ubiquitous, and because the selective pressure animals impose on plant fitness can be extremely strong, we cannot truly understand plant bet hedging strategies without accounting this selective force.

Chapter one is an explanation of why the Cox Proportional Hazard (CPH) model is an excellent choice for analyzing germination data. Historically, germination data was analyzed by analysis of variance, however the nature of germination data requires more complicated statistical analysis. The CPH is a suitable choice, but certain shortcomings in the standard CPH were identified (Ritz *et al.* 2013, see Appendix A). This chapter presents the use of the extended CPH (Kleinbaum and Klein 2012, see Appendix A), and demonstrates its ability to overcome the shortcomings of the standard CPH. The major strength of the extended CPH is the flexibility to statistically compare time intervals of interest to the user.

Chapter two presents an investigation into the variability in germination and dormancy among *G. ciliata* populations in Oklahoma. The germination trends that were observed in chapter one are explained in chapter two and it is shown that significant differences in dormancy exist among populations. Such variation may strongly affect how seedbank and population dynamics.

Chapter three is an investigation of how reproductive bet-hedging in an Oklahoma native forb *Grindelia ciliata* (Asteraceae), is affected by the seed herbivore *Schinia mortua* (Noctuidae). Female *S. mortua* moths determine the time, location, and abundance of *S. mortua* larvae that will consume the *G. ciliata* seeds. By manipulating location, timing, and abundance of larvae I evaluate the impact that this herbivore has on plant fitness.

Chapter 1: Application of the extended Cox Proportional Hazards Model to analyze seed germination data in R

Michael Kistenmacher, University of Oklahoma, Department of Microbiology and Plant Biology 770 Van Vleet Oval, Norman, Oklahoma 73019 USA

J. Phil Gibson, University of Oklahoma, Department of Biology, 730 Van Vleet Oval, Norman, Oklahoma 73019 USA

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Chapter 1: Abstract

Premise of the study: The experimental design of seed germination studies are well standardized. However, appropriate statistical analysis is not. Survival analyses, such as the Cox-Proportional Hazards model (CPH), are appropriate for analyzing germination data, but they are not commonly used because the biological interpretation of the statistical output (the Hazard Ratio) is not straightforward. We present code in R comparing a standard CPH to an extended CPH to demonstrate that the biological interpretation of the extended CPH output is superior to the standard CPH output.

Methods: Germination data were obtained from a study conducted at the University of Oklahoma. We present the nature of our germination experiment, data formatting, and preparation for analysis. We then present code for the standard CPH followed by the extended CPH, and review interpretation of each statistical output.

Results: The standard CPH derives a Hazard Ratio (*HR*) and germination probability for the two compared groups that is representative of the entire germination experiment. In contrast, the extended CPH derives *HR* and germination probabilities at specific time intervals, defined by the user, during the germination experiment.

Discussion: The extended CPH allows the user to define which time intervals to statistically compare, which is not possible using the standard CPH. This ability allows users to extract or omit information from time intervals of their choosing, such as lag periods, periods of rapid germination, or the interval during which 50% germination occurred. Therefore, researchers are able to increase description precision of temporal patterns of germination.

Chapter 1: Introduction

Seed germination studies are commonly conducted for a variety of educational levels and research purposes. Most technical experimental design issues, such as sample size, stratification treatment, or light cycles, have been established and standardized to ensure quality data recovery (Baskin and Baskin 2014). However, statistical analysis of germination data is less standardized across studies. A wide range of statistical techniques, such as analysis of variance, nonlinear regression, and generalized linear mixed models (Scott et al 1984) are typically used. However, not all of these methods are statistically appropriate in many instances because germination data include repeated measures on the same individuals (seeds), and observations are censored because of the time intervals between observations and the fraction of viable seeds that

are left ungerminated at the completion of the germination trial (Onofri 2010, McNair et al 2012).

Similar challenges were identified in medical studies where techniques called "survival analyses" have been developed to account for these critical statistical issues. Although multiple studies have indicated the usefulness of this method for germination studies (Scott et al 1984, Onofri 2010, McNair et al 2012), the application of survival analyses to germination studies has still not taken a strong foothold. There are a variety of survival analyses methods and approached, each having specific strengths and weakness (Kleinbaum and Klein 2012, McNair 2012), but the semi-parametric Cox Proportional Hazard model (CPH) (Cox 1984) is an ideal candidate when the desired goal is to compare the temporal pattern of germination or to compare time specific germination patterns among different groups (McNair et al 2012). Unfortunately, minor shortcomings of the CPH have prevented wide spread adoption. The primary complaint is the lack of convenient biological interpretation of the primary statistic, the Hazard Ratio (*HR*) (Ritz et al 2012). Additionally, the *HR* generated by the standard CPH reflects germination rates from the entire germination curve, which lumps together critical information, such as times of rapid germination and lag periods, about different stages of the germination curve. However, the extended CPH can eliminate this problem through the use of Heaviside functions, which enhances interpretation and accuracy by allowing the user to generate HR's for specific time intervals from the germination curve. The use of the extended CPH is described in Kleinbaum and Klein (2012). However that example demonstrates limited use of the Heaviside functions and does not explain the application of this technique to germination data (Kistenmacher and Gibson

2016). The goal of this paper is to demonstrate the use, advantages, and flexibility the extended CPH model has over the standard CPH for analyzing germination data in R programming software.

Chapter 1: Methods

The extended CPH model utilizes Heaviside functions to calculate the conditional probability of germination occurring through calculation of *HR* within a specified time-interval of each germination trial (Cox 1984, Kleinbaum and Klein 2012). The *HR*-value is the ratio between the germination rates *h*() of seeds in different treatments (*A* = *Treatment* and *B* = *Control*) so that,

(1) $$HR = h(A)/h(B)$$

HR indicates the conditional probability of germination for one achene type or treatment relative to another during that time interval given that germination had not happened previously (Kleinbaum and Klein, 2012; Kistenmacher and Gibson 2016). For example, if $h(A) = 1$ and $h(B) = 1$ then $HR = 1$, indicating no difference in the likelihood of seed germination during that time interval between the two groups. However, when $h(A) = 3$ and $h(B) = 1$, then $HR = 3$, then the rate of germination in the treatment group $h(A)$ is larger than that of the control group $h(B)$. This indicates a higher rate of germination in the treatment group. Interpretation of the *HR* statistic can be clarified by converting it into the probability of germination in the stored group relative to the control over a

given time period (Spruance et al 2004). Conditional probability of germination (*P*) is calculated as,

(2) $$P = HR/(1 + HR)$$

Thus, if *HR* = 1, then *P* = 0.50 or even probability of germination for the control and treatment group during that time interval. If h(*A*) = 3 and h(*B*) = 1, then *HR* = 3 and *P* = 0.75 indicating a 75% probability of the treatment group germinating before the control during that time interval. It is important to note that *HR*-values and *P*-values indicate conditional probability of germination during a given period of time, and neither reports time to germination or number of seeds germinating.

Study species and experimental design

In developing this application of the extended CPH model, we used data from *Grindelia squarrosa* (Asteraceae) achenes that were collected from a populations North of Boulder, Colorado, 40°06'16.9"N 105°16'52.0"W. *Grindelia squarrosa* exhibits cryptic heterocarpy, where each individual produces two fruit types (achenes) that are similar morphologically, but differ in germination and dormancy. Like most members of the Asteraceae, *G. squarrosa* individuals produce capitula containing centrally located disc florets and peripherally located ray florets. Disc floret ovaries develop into single seeded disc achenes, whereas ray floret ovaries develop into single seeded ray achenes. Typically, disc achenes are less dormant, and have a larger seed and thinner pericarp than ray achenes (McDonough 1975).

Achenes were collected from 20 individuals on September 2012. Achenes were returned to the lab, pooled, and sorted according to type. Germination trials consisted of three petri dishes for each achene type, containing 50 achenes of a single type that were placed on one Whatman 9 cm filter paper, were watered with 3 mL double-distilled water, and achenes were placed in a Precision Model 818 Low Temperature Illuminated Incubator (Thermo Electron Corporation®, Marietta, OH) under a decreasing temperature temperature regime (Washitani 1989, Battla and Benech-Arnold 2003), that started at 32°C, and the temperature was reduced by 4°C every 4 days for a total of 32 days (32°C, 28°C, 24°C, 20°C, 16°C, 12°C, 8°C, 4°C). Germination was scored every four days. At the end of the regime, all petri dishes were placed at 20°C for 4 days to check if non-dormant achenes remained. Germinated seeds, defined by radicle protrusion through the pericarp, were removed from the petri dishes after counting. Petri dishes were re-sealed with Parafin after each inspection. Seed viability was tested at the end of each germination trial in each ungerminated achene by removing the seed from the pericarp using a razor blade. A seed was scored as viable if the seed was white and plump (Baskin and Baskin 2014). If achenes contained nonviable seeds or no seed at the end of the germination trial, they were removed from statistical analysis.

Data format

To run an extended Cox Proportional Hazard model in R, each observation must be recorded using the start (Day1), stop (Day2), and event format (Table 1). In this example we are comparing disc and ray achenes. Because these are categorical variables, they are coded into two separate columns. In contrast, continuous variables should be assigned a unique numerical value in a single column. The “Disc” column

contains only 0's and is therefore considered as an informal control that the other group is compared against. The "Ray" column contains 0's for rows that correspond to disc achene observations, and 1's in rows that correspond to ray achene observations. An "Individual" and "replicate" columns are included to track each individual achene and petri-dish replicate. The "Event" column is where information regarding germination is recorded. During time intervals when no germination occurred, the event column is coded as 0. When germination did occur, the time interval is coded as 1. Therefore, a disc achene that germinated between days 4 and 8 will have two lines in the data set since there are only two observations, between days 0 and 4 and between days 4 and 8, for this particular seed (Table 1). In contrast, a ray achene that remained ungerminated through the end of the experiment will have nine lines in the data set (Table 1). Thus, each line of data refers to an observation made at a particular time interval.

#R code for comparing disc and ray achenes

```
#Required packages
library(survival)
library(Hmisc)
library(plotrix)
library(MASS)
#Read-in data file
germ<-read.table("SquarrosaData",header=TRUE)
#Run Kaplan-meier test to derive germination percent at each time interval.
surv.SQ<-survfit(Surv(Day1,Day2,Event)~Ray,data=germ)
```

#review data

summary(surv.SQ)

#code for visualizing disc versus ray achene comparison in supplemental material.

#Visual check for crossing of germination curves is an important way to check the

#proportional hazard assumption

#Running a standard Cox Proportional Hazard model

Y30=Surv(germ$Day1, germ$Day2, germ$Event)

Cox.SQ<-coxph(Y30 ~ Ray+cluster(Individual)+cluster(Rep), data=germ)

#check the proportional hazard assumption using a statistical test.

cox.zph(Cox.SQ,transform=rank)

#call statistical output

summary(Cox.SQ)

#Running the extended Cox Proportional Hazard model with Heaviside functions every 4 days.

pops.cph30=survSplit(Surv(Day1,Day2,Event)~Ray,germ,cut=c(4,8,12,16,20,24,28,32)

,end="Day2", event="Event",start="Day1")

pops.cph30$hvR32=pops.cph30$Ray(pops.cph30$Day1<=2)*

pops.cph30$hvR28=pops.cph30$Ray(pops.cph30$Day1>2&pops.cph30$Day1<=6)*

pops.cph30$hvR24=pops.cph30$Ray(pops.cph30$Day1>6&pops.cph30$Day1<=10)*

pops.cph30$hvR20=pops.cph30$Ray(pops.cph30$Day1>10&pops.cph30$Day1<=14*

)

```
pops.cph30$hvR16=pops.cph30$Ray*(pops.cph30$Day1>14&pops.cph30$Day1<=18
)
pops.cph30$hvR12=pops.cph30$Ray*(pops.cph30$Day1>18&pops.cph30$Day1<=22
)
pops.cph30$hvR8=pops.cph30$Ray*(pops.cph30$Day1>22&pops.cph30$Day1<=26)
pops.cph30$hvR4=pops.cph30$Ray*(pops.cph30$Day1>26&pops.cph30$Day1<=30)
pops.cph30$hvRe20=pops.cph30$Ray*(pops.cph30$Day1>30)
pops.cph30[,14]<-germ[,1]
pops.cph30[,15]<-germ[,2]
colnames(pops.cph30)=c("Ray","Day1","Day2","Event","hvR32","hvR28","hvR24","hv
R20","hvR16","hvR12","hvR8","hvR4","hvRe20","Individual","Rep")
Y30=Surv(pops.cph30$Day1,pops.cph30$Day2, pops.cph30$Event)
Cox.H.SQ<-coxph(Y30 ~ hvR32 + hvR28 + hvR24 + hvR20 + hvR16 + hvR12 + hvR8
+ hvR4 + hvRe20 + cluster(Rep) + cluster(Individual), data=pops.cph30)
 summary(Cox.H.SQ)
```

Chapter 1: Results

The statistical output generated in R (Figure 1 and Figure 2), was modified to show only essential components. Table 2 and Table 3 also contain column identifiers to simplify explanation. Column A (Table 2) contains the HR. For the standard model, HR = 0.2739, which indicates that the germination rate of ray achenes is lower than that of disc achenes. This HR is used to derive *P,* and clarifies the conditional probability of a ray achene germinating before a disc achene. In this case, ray achenes have a 21.5%

probability (P = 0.215) of germinating before disc achenes. Columns B and C provide the 95% confidence intervals (CI) of the HR value (Table 2). These 95% CI's can also be converted to P and if these probability confidence intervals do not cross 0.5, then the germination rates are significantly different. Column D contains the inverse of the HR from column A (Table 2), in other words, the HR representing the likelihood that disc achenes will germinate before ray achenes. The P calculated from the HR in column D equals 0.785 (i.e., 1 minus the P calculated from the HR in column A). The z-score and p-values are in columns E and F, respectively (Table 2).

The output of the extended CPH model contains the same variables as the standard CPH, but now each variable is available for each of the Heaviside function time intervals (Table 3). Note that all HR's in column A are less than 1 which indicates that the germination likelihood of ray achenes is lower in comparison to disc achenes in all time periods. However, not all time periods were significantly lower (Table 3). Only hvR32, hvR24, hvR20, hvR12, hvRe20 were significantly lower, but hvR28, hvR16, hvR8, and hvR4 were not significantly different (Table 3 column F). For hvR32, HR = 1.253^{-7} (Table 3), which converts to $P < 0.0001$, which means that ray achene germination during that period is extremely unlikely to occur before disc achenes. This result is driven by a small fraction of germinated disc achenes in comparison to zero ray achenes (Figure 3). For hvR28, the probability of ray achene germination was P = 0.29, which was not significantly different from disc achenes (Table 3, Figure 3). On the other hand, during hvR24 and hvR20 the probability of ray achenes germinating before disc achenes were P = 0.09 and P = 0.22, respectively. The mean germination percent during hvR24 was 30% for disc and 4% for ray achenes, and during hvR20 were 21%

for disc and 10% for ray achenes. Ray achene germination rates were significantly lower in comparison to disc achenes during both hvR24 and hvR20 (Table 3, Figure 3). Large germination fractions occurred in disc and ray achenes during hvR16, which resulted in a non-significant difference between the two groups. During hvR12, disc and ray achene germination began to plateau, although the ray achene germination probability was significantly lower (Table 3). During hvR8 and hvR4 disc and ray achene germination continued to plateau although germination occurred at about equal rates (Table 3, Figure 3). During the hvRe20 interval, the probability of ray achene germination was significantly lower than disc achenes with a probability of $P = 0.12$ (Table 3).

Chapter 1: Discussion

We demonstrate that the extended Cox Proportional Hazard (CPH) model is more versatile and powerful for the analysis of seed germination data in comparison to the more commonly used standard CPH model. In our example, both the standard and extended CPH models show that the probability of germination was lower for ray achenes compared to disc achenes (Figure 1). However the amount of information obtained from the extended cox model far exceeds the standard CPH model. A particular strength of the extended CPH is the flexibility to specify Heaviside functions that span time intervals of interest to the researcher. In our case the Heaviside functions reflected 4-day time intervals due to our experimental design, however other studies may chose different time interval durations. These Heaviside functions enabled us to determine exactly at which temperatures significant differences in germination percent

occurred, and better understand how these two achene types may differ physiologically and ecologically.

To alter Heaviside function intervals, users can modify the code containing the *survSplit()* function, and the code thereafter. In *survSplit()*, the user must select other time intervals at which to cut the dataset (A). Then, the user must create new Heaviside objects in the new dataset created by *survSplit()* (Appendix A).

There are three challenges that are important to consider when applying CPH models to analyze germination data. The first challenge occurs when more than two groups are compared and none of those groups serve the role of a control. For example, when comparing populations, the goal is often to compare all populations to each other and not to only compare them to one (control) population. However, the CPH model always treats the group with 0's in each row of the raw data file as the comparative standard (i.e., control) and compares all other groups to it. Therefore, to compare the populations that did not contain only 0's in their rows, the raw data file has to be edited so that all rows for one of those populations contain only 0's. This adds additional data management time to the process of data analysis. The second challenge is when a CPH model is comparing three or more groups. In that case, users must always include all groups or Heaviside functions on the right side of ~ in the *coxph()* function. Otherwise, the observations that belong to the group that is not referenced will be included as observations belonging to the group that is coded as all 0's. The third challenge is the recognition that *HR* and *P* by themselves do not convey any information about germination percentages. For example, the probability of ray achenes germinating before disc achenes during HvR4 was $P = 0.31$ whereas during HvRe20 $P = 0.12$ (Table

3). This may lead to the incorrect conclusion that a larger percent of ray achenes germinated during HvR4 than during HvRe20. Actually, the percent of ray achenes that germinated during hvR4 was lower (1.8%) in comparison to hvRe20 (3.7%). This occurs because the derivation of *P* is dependent on disc achene germination during that time interval. In this case, the disc achene germination percent was lower during hvR4 (4.1%) than in hvRe20 (23.9%).

The *HR* generated by the CPH is not easily interpretable for germination studies, however after converting it to *P* interpretation becomes clear. The usefulness of *P* increases when using the extended CPH, because users are able to calculate *P* for time intervals of their choosing. For example, a user can specify and compare the time interval during which germination was most rapid or during the lag periods before or after rapid germination. In addition, the extended CHP allows the user to define the time period during which a particular percentage of germination occurred and determine if the probability of germination during that time period differs for different seed lots, species, or populations. This technique can be applied to any germination study and the R-code provided in this paper provides the framework for creating custom Heaviside functions and implementing them in the extended CPH to meet the needs defined by the user.

Chapter 1: References

Baskin J.M and C.C. Baskin (2014) *Seeds: Ecology, Biogeography, and Evolution of dormancy and germination*. Second Edition, Academis Press

Batlla D., R. L. Benech-Arnold. 2003. A quantitative analysis of dormancy loss dynamics in *Polygonum aviculare L.* seeds. Development of a thermal time

model based on changes in seed population thermal parameters. *Seed Sci Res* 13:55–68

Cox, D. R. and D. Oakes. 1984. Analysis of Survival Data. London: Chapman and Hall

Kistenmacher M. and J. P. Gibson. 2016. Bet-hedging against larval herbivory and seed bank mortality in the evolution of heterocarpy. *American Journal of Botany.* 103(8): 1-13.

Kleinbaum D. G. and M. Klein. 2012. *Survival Analysis*. Springer Science Inc., New York.

McDonough N. T. 1975. Germination Polymorphism in *Grindelia squarrosa* (Pursh) Dunal. *Northwest Science* 49: 190–200.

McNair J., Sunkara A., and Forbish D., 2013. How to Analyse Seed Germination Data Using Statistical Timeto- event Analysis: Non-parametric and Semi-parametric Methods. *Peer-reviewed scientific publications*. Paper 4. http://scholarworks.gvsu.edu/peerscipub/4

Onofri A., F. Gresta and F. Tei. (2010) A new method for the analysis of germination and emergence data of weed species. *Weed Research* 50, 187–198.

Ritz C., C. B. Pipper, J. C. Steibig. 2013. Analysis of germination data from agricultural experiments. *European Journal of Agronomy* 45, 1-6.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Scott S., R. Jones, and W. Williams. 1984. Review of data analysis methods for seed germination. *Crop Science* 24, 1192–1199.

Spruance S. L., Reid J., Grace M., Samore M. 2004. Hazard ratio in clinical trials. *Antimicrobial Agents and Chemotherapy.* 48(8): 2787-2792

Washitani I. 1987. A convenient screening test system and a model for thermal germination responses of wild plant seeds: behavior of model and real seed in the system. *Plant Cell Environ.* 10:587-598.

Chapter 1: Tables

Table 1. The required data format for running an extended Cox Proportional Hazard Model in R. The grey shaded region shows two observations of one *Grindelia squarrosa* (Asteraceae) disc achene and the nonshaded region shows nine observations of a *G. squarrosa* ray achene.

Individual	Replicate	Ray	Disc	Day1	Day2	Event
2038	34	0	0	0	4	0
2038	34	0	0	4	8	1
1776	15	1	0	0	4	0
1776	15	1	0	4	8	0
1776	15	1	0	8	12	0
1776	15	1	0	12	16	0
1776	15	1	0	16	20	0
1776	15	1	0	20	24	0
1776	15	1	0	24	28	0
1776	15	1	0	28	32	0
1776	15	1	0	32	36	0

Table 2. Modified statistical output format of the standard Cox Proportional Hazard model run in R-Programming. Column A contains the Hazard Ratio (*HR*), columns B and C the upper and lower 95% confidence intervals, column D the inverse of the *HR*, column E the z-score, and column F the p-value.

	A	B	C	D	E	F
	exp(coef)	lower .95	upper .95	exp(-coef)	z	Pr(>|z|)
Ray	0.2739	0.2121	0.3537	3.651	-9.926	<2e-16 ***

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 3. Modified statistical output format of the extended Cox Proportional Hazard model run with 9 Heaviside functions in R-Programming. Column A contains the Hazard Ratio (*HR*), columns B and C the upper and lower 95% confidence intervals, column D the inverse of the *HR*, column E the z-score, and column F the p-value.

	A	B	C	D	E	F
	exp(coef)	lower .95	upper .95	exp(-coef)	z	Pr(>|z|)
hvR32	1.253e-07	5.896e-08	2.662e-07	7.982e+06	-41.326	< 2e-16 ***
hvR28	4.054e-01	1.497e-01	1.098e+00	2.466	-1.776	0.075748 .
hvR24	1.006e-01	4.639e-02	2.181e-01	9.941	-5.817	5.99e-09 ***
hvR20	2.814e-01	1.646e-01	4.809e-01	3.554	-4.637	3.53e-06 ***
hvR16	6.331e-01	3.843e-01	1.043e+00	1.579	-1.795	0.072674 .
hvR12	1.379e-01	4.617e-02	4.121e-01	7.249	-3.547	0.000389 ***
hvR8	4.131e-01	1.504e-01	1.135e+00	2.421	-1.715	0.086416 .
hvR4	4.457e-01	6.325e-02	3.141e+00	2.244	-0.811	0.417268
hvRe20	1.408e-01	4.496e-02	4.409e-01	7.103	-3.366	0.000762 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Chapter 1: Figures

```
Call:
coxph(formula = Y30 ~ Ray + cluster(Individual) + cluster(Rep),
    data = germ)

  n= 2539, number of events= 300

       coef exp(coef) se(coef) robust se      z Pr(>|z|)
Ray -1.2949    0.2739   0.1339    0.1305 -9.926   <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

    exp(coef) exp(-coef) lower .95 upper .95
Ray    0.2739      3.651    0.2121    0.3537

Concordance= 0.666  (se = 0.018 )
Rsquare= 0.042   (max possible= 0.735 )
Likelihood ratio test= 108.5  on 1 df,   p=0
Wald test            = 98.53  on 1 df,   p=0
Score (logrank) test = 106.1  on 1 df,   p=0,   Robust = 110.9  p=0

  (Note: the likelihood ratio and score tests assume independence of
     observations within a cluster, the Wald and robust score tests do not).
```

Figure 1. Statistical output generated from the standard Cox Proportional Hazard model in R-programming. The test compares *Grindelia squarrosa* (Asteraceae) disc and ray achenes.

```
Call:
coxph(formula = Y30 ~ hvR32 + hvR28 + hvR24 + hvR20 + hvR16 +
    hvR12 + hvR8 + hvR4 + hvRe20 + cluster(Rep) + cluster(Individual),
    data = pops.cph30)

  n= 2539, number of events= 300

             coef  exp(coef)   se(coef)  robust se       z Pr(>|z|)
hvR32  -1.589e+01  1.253e-07  1.276e+03  3.846e-01 -41.326  < 2e-16 ***
hvR28  -9.028e-01  4.054e-01  5.088e-01  5.084e-01  -1.776 0.075748 .
hvR24  -2.297e+00  1.006e-01  3.945e-01  3.948e-01  -5.817 5.99e-09 ***
hvR20  -1.268e+00  2.814e-01  2.737e-01  2.734e-01  -4.637 3.53e-06 ***
hvR16  -4.571e-01  6.331e-01  2.562e-01  2.547e-01  -1.795 0.072674 .
hvR12  -1.981e+00  1.379e-01  5.592e-01  5.584e-01  -3.547 0.000389 ***
hvR8   -8.840e-01  4.131e-01  5.176e-01  5.156e-01  -1.715 0.086416 .
hvR4   -8.081e-01  4.457e-01  1.000e+00  9.962e-01  -0.811 0.417268
hvRe20 -1.961e+00  1.408e-01  5.841e-01  5.824e-01  -3.366 0.000762 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

       exp(coef) exp(-coef) lower .95 upper .95
hvR32  1.253e-07  7.982e+06 5.896e-08 2.662e-07
hvR28  4.054e-01  2.466e+00 1.497e-01 1.098e+00
hvR24  1.006e-01  9.941e+00 4.639e-02 2.181e-01
hvR20  2.814e-01  3.554e+00 1.646e-01 4.809e-01
hvR16  6.331e-01  1.579e+00 3.843e-01 1.043e+00
hvR12  1.379e-01  7.249e+00 4.617e-02 4.121e-01
hvR8   4.131e-01  2.421e+00 1.504e-01 1.135e+00
hvR4   4.457e-01  2.244e+00 6.325e-02 3.141e+00
hvRe20 1.408e-01  7.103e+00 4.496e-02 4.409e-01

Concordance= 0.666  (se = 0.018 )
Rsquare= 0.052   (max possible= 0.735 )
Likelihood ratio test= 134.3  on 9 df,   p=0
Wald test            = 1797  on 9 df,   p=0
Score (logrank) test = 124  on 9 df,   p=0,   Robust = 126  p=0

  (Note: the likelihood ratio and score tests assume independence of
     observations within a cluster, the Wald and robust score tests do not).
```

Figure 2. Statistical output generated from the extended Cox Proportional Hazard model in R-programming. The test compares *Grindelia squarrosa* (Asteraceae) disc and ray achenes germination rates at 4-day intervals. Each HvR represents a Heaviside function for a different 4-day time interval.

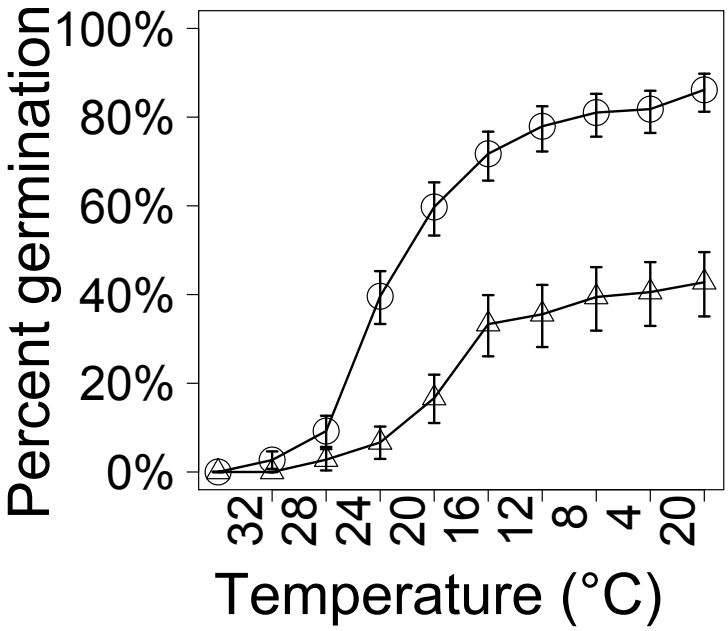

Figure 3. Mean percent germination of *Grindelia squarrosa* (Asteraceae) disc (open circle) and ray (open triangle) achenes during a decreasing temperature regime. Error bars indicate 95% confidence intervals. All ungerminated achenes at the end of the germination trial were viable.

Chapter 2: Seasonal dormancy cycling in heterocarpic *Grindelia ciliata* (Asteraceae) achenes

Michael Kistenmacher, University of Oklahoma, Department of Microbiology and Plant Biology 770 Van Vleet Oval, Norman, Oklahoma 73019 USA

J. Phil Gibson, University of Oklahoma, Department of Biology, 730 Van Vleet Oval, Norman, Oklahoma 73019 USA

Keywords: Heterocarpy; germination; Asteraceae; seed dormancy; dormancy cycling; germination cueing

Chapter 2: Abstract

Heterocarpy is an adaptive bet-hedging strategy that has evolved several times in the Asteraceae. Although the role of morphological features has been studied for several species, the dormancy changes that seeds inside the achenes undergo while ungerminated in the seedbank are not understood. Dormancy changes after dispersal may further enhance the benefits and drive evolution of bet-hedging via heterocarpy In this study we investigate the dormancy states of three achene morphs produced by *Grindelia ciliata* (Asteraceae) when fresh and after storage for 30-days and 60-days in simulated winter and summer soil temperatures to compare dormancy changes among their seeds. We found that disc achenes are almost completely nondormant and their dormancy state remained unchanged after 30 and 60 days of storage. In contrast,

intermediate and ray achenes were conditionally dormant and showed enhanced germination after exposure to warm temperatures and induced dormancy after exposure to cold temperatures. Significant differences in achene dormancy and germination behavior were detected among populations. This study shows that in addition to differences is seed protection and dispersal due to structural variation among achene morphs, *G. ciliata* achieves additional benefits of bet-hedging via germination cueing. This provides a mechanism for multiple flushes of germination from the seed bank throughout the year in response to changing environmental conditions.

Chapter 2: Introduction

Heterocarpy is a reproductive bet-hedging life-history strategy that results when one plant produces two or more dissimilar fruits with different dispersal and germination features (reviewed in Imbert 2002). Although relatively uncommon in flowering plants, heterocarpy has evolved several times in the Asteraceae due to developmental and structural features of the capitulum (Burtt 1977). In the Asteraceae, heterocarpy typically occurs between the single-seeded achenes produced by florets in central versus peripheral positions on the capitulum. Achenes from florets in the central position are characteristically nondormant and often retain functioning dispersal structures such as a pappus. In contrast, achenes produced in more peripheral locations are dormant and do not retain a functioning pappus. These differences are typically between central disc and peripheral ray florets, but heterocarpy also occurs in species that produce a single floret morph.

Structural differences between heterocarpic achenes result in bet-hedging that offsets mortality due to different predictable and unpredictable herbivory, dormancy, and dispersal risks (Forsyth and Brown 1982; Tanowitz et al 1987; McEvoy 1984; Flint and Palmbald 1978; McDonough 1975; Venable 1985b; Venable and Levin 1985; Gibson 2001; Baskin and Baskin 2013; Kistenmacher and Gibson 2016). However, there is not a clear consensus on whether germination differences among achene morphs are due to structural variation alone or differences in dormancy status of their seeds. Likewise, it is not known whether dormancy status of seeds in different achene morphs is constant or varies among achene morphs after dispersal (Baskin and Baskin 1976, Hensen 1999, Brändel 2007, Sun et al 2009, Aguado et al 2011, Puglia et al 2015). Studies of heterocarpic species native to desert environment have shown that a fraction of the dormant achene morphs and can germinate immediately when conditions become favorable thereby employing an opportunistic dormancy strategy (Venable 1987, Imbert et al 1996, Sun et al 2009, Baskin et al 2014). However, the fraction of achenes that are in such an opportunistic state vary among populations, and populations from more xeric environments tend to have smaller fractions of opportunistic achenes than populations from mesic environments (Philippi 1993). This study examines five populations located along a natural precipitation gradient in the short and mixed grass prairies of the South-central United States of America to test whether dormancy and germination features of seeds in the dramatically different achene morphs are uniform or whether they vary among achene morphs, populations, and seasons.

We investigated dormancy of heterocarpic *Grindelia ciliata* (Astereae, Asteraceae) achenes. In each capitulum, this species produces two or three achene

morphs that display extreme morphological variation among them (Gibson 2001; Kistenmacher and Gibson, 2016). Disc achenes, produced by centrally located disc florets, have large seeds, thin pericarps, and retain a functioning pappus for dispersal (Gibson 2001, Kistenmacher and Gibson 2016). In contrast, ray florets produced around the periphery of the capitulum produce ray achenes that have smaller seeds than disc florets but produce a thick lignified pericarp and lack a functional pappus (Gibson 2001, Kistenmacher and Gibson 2016). In some instances, several ranks of disc florets in positions adjacent to ray florets produce a third morph we designate as intermediate achenes. This morph has small seeds and a thick pericarp like ray achenes, but retain a functional pappus like disc achenes. Because the pericarp and seed function as a single, dispersal and germination diaspore, we use the term achene to collectively refer to the pericarp and the seed within it (Kistenmacher and Gibson 2016). Achenes mature and disperse in autumn. All disc achenes germinate readily after dispersal in late summer and autumn, whereas a majority of intermediate and ray achenes remain dormant in the seed bank and germinate in summer and autumn approximately one year after maturation and dispersal (Kistenmacher and Gibson 2016). In this study we test whether disc, intermediate, and ray achenes remain in a steady state of dormancy or if they undergo dormancy cycling. If *G. ciliata* achenes undergo dormancy cycling, exposure to cold soil temperatures should induce dormancy and prevent germination whereas exposure to warm soil temperatures will break dormancy or otherwise promote seed germination.

Chapter 2: Methods

Achene collection and germination — We collected capitula from 15 *Grindelia ciliata* individuals in each of five populations on October 31, 2012 (Table 1). Two populations were located in the North-western Panhandle state division of Oklahoma, which experience significantly lower precipitation and soil moisture levels than the statewide average, and three populations in the north-central state division (Figure 1), which typically experience soil moisture levels equal to the state average (Illston et al 2004). We chose populations at similar latitude to minimize any effects of photoperiod that have potentially shaped population seed germination characteristics (Baskin and Baskin 2014). On the same day as collection, achenes were returned to the lab, pooled by population, and sorted by achene type. The following day, fresh achenes were immediately placed into incubators to start the initial germination trial and establish the baseline level of dormancy and germination response to increasing and decreasing temperatures.

Germination trials were conducted in Precision Model 818 Low Temperature Illuminated Incubators (Thermo Electron Corporation®, Marietta, OH) at temperatures mimicking increasing spring soil temperatures and decreasing autumn soil temperatures of autumn to recreate an ecologically relevant range of temperatures that reflect average Oklahoma soil temperatures following summer and winter (McPherson et al 2007, Brock et al 1995, Figure 2). Initial germination trials for each achene type from each population consisted of six petri dishes containing 25 achenes each of a single morph. Achenes were placed on a single disc of Whatman 9 cm filter paper and hydrated with 3 mL double-distilled water. Three dishes were placed in an increasing temperature

regime (ITR) and three were places in a decreasing temperature regime (DTR) (Washitani 1989, Battla and Benech-Arnold 2003). The DTR started at 32°C, and the temperature was reduced by 4°C every 4 days for a total of 32 days until reaching a final temperature of 4°C (32°C, 28°C, 24°C, 20°C, 16°C, 12°C, 8°C, 4°C). The ITR started at 4°C, and the temperature was increased by 4°C every 4 days for a total of 32 days until reaching a final temperature of 32°C (4°C, 8°C, 12°C, 16°C, 20°C, 24°C, 28°C, 32°C). Germination, defined by radicle protrusion through the pericarp, was scored on the second and fourth day at each temperature. Germinated seeds were removed from the petri dishes after counting and petri dishes were re-sealed with Parafin after each inspection. Seed viability of each ungerminated achene was tested at the end of each germination trial by removing the seed from the pericarp using a razor blade. A seed was scored as viable if the seed was white and plump (Baskin and Baskin 2014). Achenes containing nonviable seeds or no seed at the end of the germination trial, they were removed from statistical analysis.

The remaining *G. ciliata* achenes were dry-stored in manila coin envelopes for 30 and 60 days under either a constant 5°C to replicate cold dry after-ripening (CDAR) under cold winter soil temperatures or under a constant 27.5°C in a Precision Model 818 Low Temperature Illuminated Incubator (Thermo Electron Corporation®, Marietta, OH) to replicate warm dry after-ripening (WDAR) under warm summer soil temperatures. Achenes were dry-stored because disc achenes germinate immediately once hydrated. After storage, we conducted germination trials on WDAR achenes under DTR conditions to simulate germination and dormancy under decreasing soil temperatures following exposure to warm soil seedbank temperatures in late summer

and autumn. Conversely, CDAR achenes were placed in a germination trial under ITR conditions to simulate their dormancy and germination in response to increasing soil temperatures after overwintering in the soil seed bank. These scenarios replicate natural soil temperature conditions (Figure 2). Due to unexpected problems during the experiment, 30-day storage treatment data are not available for P1 disc, intermediate, and ray achenes, and for NC1 intermediate achenes in the DTR.

Statistical Analysis—We calculated the Hazard Ratio (*HR*) through an Extended Cox Proportional Hazard Model (CPH) with Heaviside functions to compare germination among achene types, populations, and storage treatments, (McNair *et al.* 2013, Kistenmacher and Gibson 2016) using R version 3.1.0 (R Development Core Team 2014). The extended CPH model utilizes heaviside functions to calculate the conditional probability of germination occurring within a specified time-interval of a germination trial through calculation of *HR* (Cox 1984, Kleinbaum and Klein 2012). The *HR*-value is the ratio between the germination rates of seeds in different treatments, and, therefore, indicates the conditional probability of germination for one achene type or treatment relative to another during that time interval given that germination had not happened previously (Kleinbaum and Klein, 2012; Kistenmacher and Gibson 2016). In this paper, we always present the *HR* so that the control germination rate (i.e., fresh seeds) is always in the denominator and treatment group germination rate is always in the numerator. For example, if only one stored and one fresh seed germinate over a given time interval, then $HR = 1$ and there is no difference in the likelihood of seed germination during that time interval between the two groups. However, if 20 stored

seeds and only one fresh seed germinates, then *HR* > 1 which indicates that stored seeds are more likely to germinate than fresh seeds. Interpretation of the *HR* statistic is clearer when it is converted into the probability of germination (Spruance 2004). The probability of germination (*P*) is calculated as,

$$P = HR/(1 + HR) \quad (1)$$

where *HR* is the hazard ratio calculated between treatment and fresh (control) achenes over a given time period. Thus, if *HR* =1, then *P* = 0.50 and there is an even probability of germination for the control and treatment group during that time interval. If *HR* = 2, then *P* = 0.67 indicating a 67% probability of the treatment group germinating before the control during that time interval. Correspondingly, the probability that a seed in the control group germinates before the treatment group is 33%. It is important to note that *HR*-values and *P*-values indicate conditional probability of germination during a given period of time, and neither reports time to germination or number of seeds germinating.

Chapter 2: Results

Increasing temperature regime

Fresh disc achenes— Fresh disc achenes rapidly initiated germination at 8°C in all populations and overall, 69.7% ± 2.4 (mean ±standard error) of all disc achenes germinated by the end of the 8°C period (Figure 3, Figure 4). This was significantly higher than intermediate achene (*P* = 1.0, p > 0.001) and ray achene germination at 8°C (*P* = 1.0, p > 0.001). Significant differences in mean disc germination occurred among populations at 8°C (Figure 3). Probability of disc achene germination at 8°C was significantly higher in NC2 than NC3 (*P* = 2.4, p < 0.001), NC1 (*P* = 2.9, p < 0.001), P2

(P = 1.6, p = 0.007), and P1 (P = 1.9, p < 0.001). The probability of fresh disc achene germination was also significantly lower for NC1 disc achenes than in P2 (P =0.58, p=0.006, Figure 3). There were no interpopulation differences in germination of remaining achenes at higher temperatures, and all populations achieved 100% germination by the end of the fourth day at 16°C (Figure 3, Figure 4).

Fresh intermediate achenes— Fresh intermediate achene germination reached an average of only 13.7% ± 1.8 across all populations (Figure 3, Figure 4). The largest mean total germination occurred in P1 (30% ± 5.5), followed by NC3 (20.3% ± 4.8), NC2 (17.2% ± 4.7), P2 (1.4% ± 1.4), and lastly NC1 (0%) achenes (Figure 3). Germination initiated at 12°C in P1 and at 16°C in P2, NC2, and NC3 (Figure 3). Mean germination at 16°C, 20°C, and 24°C was significantly more likely in P1, NC2, and NC3 achenes in comparison to NC1 and P2 achenes (Figure 3). No significant differences in mean germination were detected among P1, NC2, and NC3 achenes at 16°C, 20°C, and 24°C (Figure 3).

Fresh ray achenes— Ray achene germination was lowest of the three achene morphs. Mean total germination across all populations only reached 5.7% ± 1.3 (Figure 4). The highest mean germination occurred in NC2 (19% ± 5.1), followed by NC1 (5.6% ± 2.7), P1 (3.4% ± 2.4), NC3 (2.9% ± 1.9), and lastly P2 (0%) (Figure 3). Ray achene germination initiated at 16°C in P1 and NC2 achenes and at 20°C in populations NC3 and NC1 (Figure 3). Ray achene germination initiated and had similar germination behavior to NC2 ray achenes at this temperature. A total of eight ray achenes germinated among P1, NC2, and NC3 at 24°C, and one final ray achene germinated in population NC2 at 28°C (Figure 3).

Decreasing temperature regime (DTR)

Fresh disc achenes— Mean total disc achene germination rapidly reached 95% ± 1.2 (mean ±standard error) within the first 4 days, at 32°C and achieved 100% in all populations shortly thereafter (Figure 3, Figure 8). All P1 disc achenes germinated at 32°C, which was significantly higher than in NC1 ($P = 0.59$, $p < 0.001$) and P2 ($P = 0.55$, $p = 0.03$) achenes (Figure 3). No significant differences in germination were detected at 28°C. P2 disc achenes finished germinating by the end of the 28°C treatment, and disc achenes from NC1 and NC2 finished germinating at 24°C. NC3 disc achenes finished germination at 20°C (Figure 3).

Fresh intermediate achenes— Mean total intermediate achene germination in the DTR was 62.8% ± 2.8 across populations (Figure 8). Intermediate achene germination was highest in population P1 (72.9% ± 6.4), followed by NC2 (66.7% ± 5.8), NC1 (55.4% ± 6.2), NC3 (45% ± 6.6), and lastly P2 achenes (42% ± 5.9). Although germination occurred at all temperatures, only NC3 intermediate achenes initiated germination at 32°C, whereas NC2 and NC1 intermediate achenes initiated germination at 28°C. Intermediate achenes from P1 and P2 achenes did not initiate germination until 24°C (Figure 3). P1 intermediate achene germination was significantly more likely at 24°C than intermediate achene germination in populations NC3 ($P = 0.89$, $p = 0.04$), NC2 ($P = 0.83$, $p = 0.04$), and P2 ($P = 0.84$, $p = 0.03$), but not NC1 ($P = 0.76$, $p = 0.09$, Figure 3). Similarly, P1 intermediate achenes were also significantly more likely to germinate at 20°C than NC3 ($P = 0.80$, $p = 0.02$), NC1 ($P = 0.78$, $p = 0.02$), and P2 ($P = 0.77$, $p = 0.02$), and at 16°C than all other populations

(Figure 3). At 12°C and 8°C, zero achenes from P1 germinated which was significantly fewer than in all other populations (Figure 3).

Fresh ray achenes—Mean total germination of fresh ray achenes reached 53.9% ± 2.7 across populations (Figure 8), and was highest in NC2 (46.6% ± 6.5) achenes, followed by P1 (46.4% ± 6), NC1 (40.8% ± 5.8), P2 (27.4% ± 5.2), and lastly NC3 achenes (19% ± 4.6). Germination initiated at 20°C in all populations except for NC3, whose ray achenes did not initiate germination until temperatures decreased to 12°C (Figure 3). Of the populations that initiated ray germination at 20°C the only significant difference was that P1 germination was larger than P2 germination (Figure 3). Also, significantly fewer P2 achenes germinated at 16°C in comparison to P1, NC1, and NC2 achenes (Figure 3).

ITR after cold dry after-ripening (CDAR)

CDAR disc achenes—As with fresh achenes, all cold stratified disc achenes initiated germination at 8°C and germination reached 100% for all populations by 16°C (Figure 4). Similarly, for each population 30-day or 60-day CDAR stored achene germination was not significantly different than fresh achenes with the exception of NC2 achenes at 8°C and 16°C (Figure 5). Fresh NC2 disc achenes had greater germination at 8°C than 30-day or 60-day CDAR, although this difference was no longer evident at the next higher temperature, 12°C (Figure 5). All fresh achenes germinated at 12°C, and a small fraction of 30-day and 60-day stored achenes germinated at 16°C.

CDAR intermediate achenes— Mean total intermediate achene germination across all populations was lower after 30-day CDAR than in fresh achenes with significant differences in germination detected at 16°C and 20°C (Figure 4). The mean intermediate achene germination was also low within populations, reaching only 1.5% ± 1.5 in P2, 1.3% ± 1.3 in NC1, 5.3% ± 2.3 in NC2, and 8% ± 3.1 in NC3 (Figure 6). Significant reduction in intermediate achene germination after CDAR occurred in P1, NC2, and NC3 achenes (Figure 6). Across all populations, 60-day CDAR also significantly lowered the mean germination probability at 16°C and 20°C (Figure 4). Mean total percent germination was also lower than fresh achene germination in each population, reaching 0% for P2 and NC1, 4.2% ± 2.3 for NC3, 4.4% ± 2.5 for NC2, and 4.7% ± 2.6 for P1 achenes (Figure 6). Significant reductions in germination were most distinct in P1 achenes at 12°C, 16°C, 24°C, and 28°C (Figure 6).

CDAR ray achenes— Ray achene germination was significantly reduced after 30-day CDAR (Figure 4). Mean total percent germination was 0% for NC1 and P2 achenes, 2.7% ± 1.9 for NC3, and 2.7% ± 1.8 for NC2 achenes (Figure 7). Significant reductions in ray achene germination after 30-day CDAR occurred in NC2 ray achenes at 20°C, 24°C and 28°C, and in NC1 achenes at 20°C and 24°C (Figure 7). Significant reduction in ray achene germination also occurred after 60-day CDAR ($P = 0.17$, $p = 0.004$). Mean total percent ray achene germination after 60-day CDAR was 0% for P1, P2, and NC1 achenes, 5.7% ± 3.2 for NC2, and 1.4% ± 1.4 for NC3. Significant reductions in ray achene germination occurred P1 achenes at 16°C and 24°C, in NC1 achenes at 20°C and 24°C, in NC2 achenes at 16°C, 24°C, and 28°C, and in NC3 at 24°C (Figure 7).

DTR after warm dry after-ripening (WDAR)

WDAR disc achenes **-** Germination of disc achenes after 30-day and 60-day WDAR was not significantly different from germination of fresh achenes (Figure 8). Germination of 30-day and 60-day WDAR disc achenes reached 100% in all populations by the end of the 20°C interval. No significant differences were observed between stored and fresh achenes in any population during the 32°C interval, when over 80% of achenes germinated (Figure 9). Thereafter, some significant differences were observed but they were all due to germination in one group being compared to zero germination in the other group (Figure 9). For example, P2 disc achene germination was significantly higher at 24°C and 20°C in the 60-day WDAR group because germination had finished in fresh achenes at 28°C (Figure 9).

WDAR intermediate achenes— Mean intermediate achene germination was significantly lower after 30-day WDAR than in fresh achenes during the 12°C, 8°C, and 4°C temperature intervals (Figure 8). However, the germination reductions were only significant in P2 achenes at 12°C and 4°C, and in NC2 achenes at 4°C (Figure 10). In contrast, achenes from NC3 showed no significant changes in germination after 30-day WDAR (Figure 10). Sixty-day WDAR significantly increased the overall probability of intermediate achene germination, at 20°C, 12°C, and 8°C (Figure 8). In comparison to fresh achenes, populations P2, NC2, and NC3 had significantly increased mean germination after the 60-day WDAR treatment (Figure 10). Intermediate achenes from population P2 showed a significant increased in germination at 24°C, 16°C, and 4°C,

NC2 achenes had increased germination at 20°C, and NC3 achenes had increased germination at 20°C, 12°C, and 8°C (Figure 10).

WDAR ray achenes— Overall ray achene germination after 30-day WDAR was not significantly more likely than fresh ray achene germination (Figure 8). In contrast, 60-day WDAR significantly increased the ray achene germination ($P = 0.67$, $p < 0.001$) at 24°C, 20°C, 16°C, 12°C, and 8°C (Figure 8). No differences in germination were detected between fresh and 30-day or 60-day WDAR ray achene germination in population P1. In contrast, 60-day WDAR significantly increased probability of ray achene germination in population P2 at 24°C, 20°C, 16°C, 12°C, 8°C (Figure 11). After 60-day WDAR, germination probability was significantly higher than for fresh ray achenes in NC1 and NC2 at 24°C, NC1 at 20°C, and NC3 at 20°C and 16°C (Figure 11).

Chapter 2: Discussion and Conclusion

In addition to extreme morphological and anatomical differences among achene morphs, we found evidence that *Grindelia ciliata* achieves further advantages of reproductive bet-hedging through differences in germination cueing among achene morphs. Bet-hedging via heterocarpy is known to be adaptive in unpredictable environments such as deserts (Venable 1985b), however in more mesic and seasonally predictable environments such as prairies, the evolution of germination cueing is adaptive and can provide further benefits in a heterocarpic system (Baskin and Baskin 1976, Brändel 2007, Donohue et al 2010). We found that *G. ciliata* disc achenes from all populations were unaffected by storage at average winter (CDAR) or summer

(WDAR) soil temperatures. These achenes do not undergo germination cueing and function to germinate immediately upon experiencing favorable temperature and moisture conditions. In contrast, fresh intermediate and ray achenes are in a conditional state of dormancy and experience reduced germination after CDAR, indicating the induction into a deeper state of conditional dormancy. For *G. ciliata,* increase of conditional dormancy in intermediate and ray achenes due to CDAR is likely a mechanism to prevent germination during winter and spring, which are potentially unfavorable seasons for seedling survival. In contrast intermediate and ray achenes showed increased germination after WDAR, indicating loss of conditional dormancy. For *G. ciliata*, loss of conditional dormancy in intermediate and ray achenes due to WDAR increases the likelihood that achenes germinate in autumn in the same year as dispersal, and possibly one year after dispersal. If germination does not occur in autumn, it is likely that achenes regain a deeper state of conditional dormancy while experiencing winter temperatures. Therefore, all *G. ciliata* achene types are cued to germinate in autumn, which is a strong indication this is the most adaptive time for germination. It is likely that autumn germination is adaptive because seedlings experience longer exposure to elevated soil moisture conditions prior to rapid soil drying in early summer (Illston et al 2004).

A fraction of fresh intermediate (40-75%) and ray (20-50%) achenes are capable of germinating along with disc achenes in the same season as dispersal. This fraction could potentially increase in early-dispersed achenes, which may experience soil temperatures similar to the WDAR treatment (Figure 2). This strategy may be adaptive because it provides a safer recruitment opportunity in the same season as dispersal than

disc achenes due of narrower germination-inducing temperatures and more stable soil moisture level requirements. Disc achenes are a very high-risk germination strategy, because given adequate water availability, 100% of disc achenes germinate at temperatures ranging from 8°C to 32°C and possibly above 32°C. Due to thin pericarps, disc seeds imbibe rapidly and do not require prolonged elevated soil moisture levels to germinate, leaving recently germinated disc seedlings vulnerable to rapidly declining soil moisture conditions. Rapid soil drying occurs during the beginning of *G. ciliata* achene dispersal in Oklahoma (August-October) when soil temperatures are still high and soil moisture levels have not recharged following the enhanced soil drying phase that occurs during summer (Illston et al 2004). Soil moisture levels near the surface can quickly decline after precipitation events if soil moisture at greater depths is low and surface temperature is high. Intermediate and ray achenes may remain ungerminated during such highly variable soil moisture conditions because more sustained elevated soil moisture conditions may be required for germination induction since the thick pericarps reduce the rate of imbibition, as was shown in the closely related *G. squarrosa* (McDonough 1975) that exhibits a less extreme achene heteromorphism. The sustained elevated soil moisture levels required for intermediate and ray achene germination may be associated with more suitable post-germination growing conditions, which could select for the evolution of conditional dormancy in fresh intermediate and ray achenes.

The conditional dormancy of intermediate and ray achenes is an important aspect to understanding the evolutionary ecology of heterocarpy in *G. ciliata*, because conditionally dormant achenes are morphologically similar as fully dormant achenes but differ significantly in ecology. Dormant intermediate and ray achenes will likely not

germinate under any conditions in the same season as dispersal and will therefore join the longterm seedbank. The difference between conditional dormancy and full dormancy can lead to these achenes experiencing drastically different mortality risks and survival probabilities. Thus, merely counting the proportion of disc, intermediate, and ray achenes does not adequately capture the degree of risk spreading in heterocarpic species, because conditionally dormant and dormant intermediate and ray achenes are combined into the same category, and therefore overestimates the proportion of achenes allocated to the longterm seedbank or underestimates the fraction that can emerge in the same season as dispersal.

In addition to producing fresh conditionally dormant intermediate and ray achenes, *G. ciliata* individuals can offset the high disc achene mortality risks shortly after germination by spreading dispersal events over time through staggering multiple flowering events in time (Ritland 1983). Producing capitula that disperse disc achenes during early autumn, *G. ciliata* individuals can capitalize on early season germination opportunities, which can result in larger seedlings and increase seedling fitness (Lu et al 2014). Whereas, dispersing disc achenes during late autumn can avoid the uncertain soil moisture conditions of early autumn that could result in the loss of all germinated seedlings. However, *G. ciliata* individuals are not guaranteed the resources required for the production of more than one capitulum or the longevity to produce sequentially flowering capitula. Therefore, producing conditionally dormant intermediate and ray achenes in the same capitulum as disc achenes provides a same-season-as-dispersal recruitment opportunity during more favorable post-germination survival conditions, in the case that a *G. ciliata* individual only produces one capitulum.

Intermediate and ray *G. ciliata* achenes that do not germinate in the same year as dispersal likely remain ungermianted in the seedbank until spring because average winter soil temperatures are below minimum temperatures at which we observed germination initiation (8°C). In the spring, when soil temperatures increase to 16°C - 20°C, a small percent of intermediate and ray achenes could germinate, as was seen in some populations tested in this study (Figure 5 and 6), as well as field tested achenes (Kistenmacher and Gibson 2016). It is unlikely, however, that intermediate and ray achene germination occurs after soil temperatures rise above 24°C (Figure 5, Figure 6). These achenes remain ungerminated in the seedbank and consequentially experience summer soil temperatures in the seedbank. It is likely that during summer soil temperatures, these achenes enter a state of lower conditional dormancy, similar to WDAR achenes, increasing the likelihood of germinating in the fall as soil temperatures decline. The average duration of days above 25°C in Oklahoma is approximately 90 days (McPherson et al 2007, Brock et al 1995), which may relieve dormancy in all ray and intermediate achenes.

The dormancy states of fresh, and warm stored achenes varied among the sampled populations, but no clear trend between the degree of dormancy and differences in mean annual precipitation or longitude. Variability in fresh achene dormancy (Figure 3) and loss of dormancy via WDAR, suggest that these populations differ in seedbank allocations and seedling emergence dynamics, which may affect short and long term consequences of these populations. For example, a larger portion of P1 fresh intermediate and ray achenes could germinate soon after dispersal in comparison to NC3 achenes, which would result in a larger seedbank of NC3 achenes.

The consequences of allocating fewer intermediate and ray achenes to the seedbank may increase the population extinction risk or could result in more competition among related seedlings.

Many species evolve germination cueing (Baskin and Baskin 1983a, 1984a, Baskin et al 1993), however, most of them do not exhibit extreme morphological variation among fruits. Therefore, in nonheterocarpic species germination cueing is caused solely by physiological changes inside seeds (Footitt et al 2011). However for *G. ciliata*, it is not clear if germination cueing in intermediate and ray achenes is caused in part by physiological and physical factors. Other members of the Asteraceae show physiological changes in seeds during warm dry after-ripening (Baskin and Baskin 2014), suggesting that *G. ciliata* dormancy changes after WDAR are at least in part physiologically induced. The physiological condition inside intermediate and ray achenes may explain differences between fully dormant and conditionally dormant achenes, but also differences among populations. However, we are unable to determine if physiological differences in intermediate and ray achenes are caused by genetic differences among populations, environmental factors, or a combination of both. Genetically, *G. ciliata* populations are known to differ significantly (Gibson 2001), which may indicate that selection has selected for the observed dormancy characteristics in these populations. However, germination is also a very plastic trait, and is under strong influence from environmental factors (Clauss and Venable 2000, Galloway 2005, Schmitt et al 1992, Munir et al 2001, Donohue et al 2005; 2010). Therefore more controlled investigations are needed to determine to what extent genetic differentiation among populations is influencing achene dormancy. Our study shows

that although the fundamentals of heterocarpic systems are well studied, there are several aspects that are yet to be fully understood.

Chapter 2: References

Aguado M., J. J. Martınez-Sanchez, J. Reig-Arminana, F. J. Garcıa-Breijo, J. A. Franco, and M. J. Vicente. 2011. Morphology, anatomy and germination response of heteromorphic achenes of Anthemis chrysantha J. Gay (Asteraceae), a critically endangered species. *Seed Science Research*. 21. 283-294.

Baskin C. C., P. L. Chesson, and J. M. Baskin. 1993. Annual seed dormancy cycles in two desert winter annuals. *Journal of Ecology.* Vol. 81, pp 551-556.

Baskin J.M., and C.C. Baskin. 1976. Germination dimorphism in *Heterotheca subaxillaris* var. *subaxillaris*. *Bulletin of the Torrey Botanical Club. Vol 103, No5, pp 201-206.*

Baskin J. M., and C. C. Baskin. 1983a. The germination ecology of *Veronica arvensis*. *The Journal of Ecology*. Vol 71, 57-68.

Baskin J. M., and C. C. Baskin. 1983b. Seasonal changes in the germination responses of buried seeds of *Arabidopsis thaliana* and ecological interpretation. *Botanical Gazette*. Vol 144, 540-543.

Baskin J. M., and C. C. Baskin. 1984a. Effect of temperature during burial on dormant and nondormant seeds of *Lamium amplexicaule* and ecological implications. *Weed Research*. Vol 24, 333-339.

Baskin J. M., and C. C. Baskin. 1984a. Role of temperature in regulating timing of germination in soil seed reserves of *Lamium purpureum*. *Weed Research*. Vol 24, 341-349.

Baskin J. M., and C. C. Baskin. 1985. The Annual dormancy cycle of buried weed seeds: A continuum. *BioScience*. Vol 35, No. 8, pp 492-498

Baker, G. A. and D. J. O'Dowd. 1982. Effect of parent plant density on the production of achene types in the annual *Hypochoeris glabra*. *Journal of Ecology* 70: 201–215.

Baskin J.M and C.C. Baskin (2014) *Seeds: Ecology, Biogeography, and Evolution of dormancy and germination*. Second Edition, Academis Press

Baskin J.M., J.J. Lu, Baskin C.C., D. Y. Tan. 2013. The necessity for testing germination of fresh seeds in studies on diaspore heteromorphism as a life-history strategy. *Seed Science Research* 23, 83-88.

Baskin J.M., J.J. Lu, Baskin C.C., D. Y. Tan, L. Wang. 2014. Diaspore dispersal ability and dregree of dormancy in heteromorphic species of cold deserts of northwest China: A review. *Perspectives in Plant Ecology, Ecolution, and Systematics* 16, 93-99.

Batlla D., R. L. Benech-Arnold. 2003. A quantitative analysis of dormancy loss dynamics in *Polygonum aviculare L.* seeds. Development of a thermal time model based on changes in seed population thermal parameters. *Seed Sci Res* 13:55–68

Brändel M. 2004. Dormancy and germination of heteromorphic achenes of *Bidens frondosa*. Flora, 199, pp. 228–233

Brändel M. 2007. Ecology of achene dimorphism in *Leontodon saxatilis*. Annals of Botany, 100, pp. 1189–1197

Brock, F. V., K. C. Crawford, R. L. Elliott, G. W. Cuperus, S. J. Stadler, H. L. Johnson, and M. D. Eilts, 1995: The Oklahoma Mesonet: A technical overview. *J. Atmos. Oceanic Technol.*, 12, 5-19

Brown J. S. and D. L. Venable. 1986. Evolutionary ecology of seed bank annuals in temporally varying environments. *American Naturalist* 127: 31-47.

Burtt, B L. 1977. Aspects of diversification in the capitulum. In: Hey wood, V. H., Harborne, J. B. and Turner, B. L. (eds.) *The Biology and Chemistry of the Compositae*. Vol. 1. Academic Press, London, pp. 41–59.

Clauss M. J. and D. L. Venable. 2000. Seed germination in desert annuals: An empirical test of adaptive bet hedging. *American Naturalist*. Vol. 155 : 168 – 186 .

Cohen D. 1966. Optimizing reproduction in a randomly varying environment. *Journal of Theoretical Biology* 12: 119 – 129.

Cox, D. R. and D. Oakes. 1984. Analysis of Survival Data. London: Chapman and Hall

Cruz-Mazo G., Narbona E., Luisa Buide M. 2010. Germination Patterns of Dimorphic Achenes in Three Related Species of Scorzoneroides (Asteraceae, Lactuceae) Growing in Different Environments. *Annales Botanici Fennici*, 47(5): 337-345.

Dakshini K.M.M. and S.K. Aggarwal. 1974. Intracapitular cypsele dimorphism and dormancy in *Bidens bipinnata. Biol Plant* 16: 469. doi:10.1007/BF02922239

Ruiz de Clavijo E. 1995. The ecological significance of fruit heteromorphism in the amphicarpic species *Catanache lutea* (Asteraceae). International Journal of Plant Sciences, 156, pp. 824–833

Ruiz de Clavijo E. 2001. The role of dimorphic achenes in the biology of the annual weed *Leontodon longirrostris*. Weed Research, 41, pp. 275–286

Donohue K., R. R. de Casas, L. Burghardt, K. Kovach, and C. G. Willis. 2010. Germination, post germination adaptation, and species ecological ranges. *Annual Review of Ecology, Evolution, and Systematics* 41: 293–319.

Donohue K., L. Dorn, C. Griffith, E. Kim, A. Aguilera, C.R. Polisetty, and J. Schmitt. 2005. Environmental and genetic influences on the germination of Arabidopsis thaliana in the field *Evolution* 59 vol. (4); pp. 740-757

El-Keblawy A. 2003. Effects of achene dimorphism on dormancy and progeny traits in the two ephemerals Hedypnois cretica and Crepis aspera (Asteraceae). *Canadian Journal of Botany.* 81 (6), 550-559

Flint S. D. and I. G. Palmblad. 1978. Germination Dimorphism and Developmental Flexibility in the Ruderal Weed Heterotheca grandiflora. *Oecologia.* Vol. 36, No. 1, pp. 33-43

Forsyth C, Brown N.A.C. 1982. Germination of the dimorphic fruits of *Bidens pilosa* L. New Phytologist. Vol. 90 :151–164.

Footitt S., Douterelo-Soler I., Clay H., Finch-Savage W. E. 2011. Dormancy cycling in *Arabidopsis* seeds is controlled by seasonally distinct hormone-signaling pathways. *PNAS*. vol. 108, no. 50: 20236-20241

Galloway L. F. 2005. Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytologist* 166: 93-100.

Gibson J. P. 2001. Ecological and genetic comparison between ray and disc achene pools of the heteromorphic species *Prionopsis ciliata* (Asteraceae). *International Journal of Plant Sciences* 162: 137–145.

Hensen I. 1999. Life strategies of semi-arid grassland community-mechanisms of dispersal and reproduction within Lapiedro martinezii–Stipetum tenacissimae (southeastern) Spain. Feddes Repertorium, 110, pp. 265–285

Imbert E., J. Escarré, J. Lepart. 1996. Achene Dimorphism and Among-Population Variation in *Crepis sancta* (Asteraceae). *International Journal of Plant Sciences*, 157, pp. 309–315

Imbert E., J. Escarré, J. Lepart. 1999. Differentiation among populations for life history, morphology, head traits and achene morph productions in the heterocarpic species *Crepis sancta* (Asteraceae). *International Journal of Plant Sciences*, 160, pp. 543–552

Imbert E. 2002. Ecological consequences and ontogeny of seed heteromorphism. *Perspectives in Plant Ecology, Evolution and Systematics* 5: 13 – 36.

Illston B., J. Basara, and K. Crawford. 2004. Seasonal to Interannual Variations of Soil Moisture Measured in Oklahoma. *International Journal of Climatology* 24: 1883-1896

Kistenmacher M. and J. P. Gibson. 2016. Bet-hedging against larval herbivory and seed bank mortality in the evolution of heterocarpy. *American Journal of Botany.* 103(8): 1-13.

Kleinbaum D. G. and M. Klein. 2012. *Survival Analysis*. Springer Science Inc., New York.

Leverett L.D. and C. L. Jolls. 2013. Cryptic seed heteromorphism in Packera tomentosa (Asteraceae): 13 differences in mass and germination. Plant Spec. Biol.. doi: 10.1111/1442-1984.12011

Lu J. J., D. Y. Tan, J. M. Baskin, and C. C. Baskin. 2014. Germination season and watering regime, but not seed morph, affect life history traits in a cold desert diaspore-heteromorphic annual. *Plos ONE* 9(7), e102018 (2014).

Mandák B. and P. Pyšek. 2001a. Fruit dispersal and seed banks in *Atriplex sagittata*: the role of heterocarpy. *Journal of Ecology* 89: 159–165.

McDonough N. T. 1975. Germination Polymorphism in *Grindelia squarrosa* (Pursh) Dunal. *Northwest Science* 49: 190–200.

McPherson, R. A., C. A. Fiebrich, K. C. Crawford, R. L. Elliott, J. R. Kilby, D. L. Grimsley, J. E. Martinez, J. B. Basara, B. G. Illston, D. A. Morris, K. A. Kloesel, S. J. Stadler, A. D. Melvin, A.J. Sutherland, and H. Shrivastava, 2007: Statewide Monitoring of the Mesoscale Environment: A Technical Update on the Oklahoma Mesonet. J. Atmos. Oceanic Technol., 24, 301-321.

McNair J., Sunkara A., and Forbish D., 2013. How to Analyse Seed Germination Data Using Statistical Timeto- event Analysis: Non-parametric and Semi-parametric Methods. *Peer-reviewed scientific publications*. Paper 4. http://scholarworks.gvsu.edu/peerscipub/4

Munir J., LA Dorn, K Donohue and J Schmitt. 2001. The effect of maternal photoperiod on seasonal dormancy in Arabidopsis thaliana (Brassicaceae). *American Journal of Botany*. 88 vol. (7); pp. 1240-1249

Philippi, T. 1993. Bet-hedging germination of desert annuals: variation among populations and maternal effects in *Lepidium lasiocarpum. American Naturalist.* 142: 488-507

Puglia G., S. Grimaldi, A. Carta, P. Pavone, and P. Toorop. 2015. Pericarp structure of Glebionis coronaria (L.) Cass. ex Spach (Asteraceae) cypselae controls water uptake during germination', *Seed Science Research*, 25(3), pp. 255–266. doi: 10.1017/S0960258515000148.

McEvoy P. B. 1984. Dormancy and dispersal in dimorphic achenes of tansy ragwort, *Senecio jacobaea* L (Compositae). Oecologia, 61, pp. 160–168

Olivieri I., and P.H. Gouyon. 1985. Seed dimorphism for dispersal: theory and implications. *Structure and functioning of plant populations* (eds. Heak, Woldendrop, J.W.), pp. 77-90. North-Holland Publ., Amsterdam.

Onofri A., F. Gresta and F. Tei. (2010) A new method for the analysis of germination and emergence data of weed species. *Weed Research* 50, 187–198.

Ritland K. 1983. The joint evolution of seed dormancy and flowering time in annual plants living in variable environments. *Theoretical Population Biology. Vol.* 24: 213 – 243.

Ritz C., C. B. Pipper, J. C. Steibig. 2013. Analysis of germination data from agricultural experiments. *European Journal of Agronomy* 45, 1-6.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Schmitt J., J. Niles, and R. D. Wulff. 1992. Norms of reaction of seed traits to maternal environments in *Plantago lanceolata.* American Naturalist 139: 451-466.

Spruance S. L., Reid J., Grace M., Samore M. 2004. Hazard ratio in clinical trials. *Antimicrobial Agents and Chemotherapy.* 48(8): 2787-2792

Sun H. Z., Lu J. J., Tan D. Y., Baskin J. M., Baskin C. C. 2009. Dormancy and germination characteristics of the trimorphic achenes *of Garhadiolus papposus* (Asteraceae), an annual ephemeral from the Junggar Desert, China. *S Afr J Bot* 75: 537–545.

Tanowitz B. D., P. F. Salopek, and B. E. Mahall. 1987. Differential germination of ray and disc achenes in *Hemizonia increscens* (Asteraceae). American Journal of Botany, 74, pp. 303–312

Venable D. L. 1985b. Ecology of achene dimorphism in *Heterotheca latifolia* III. Consequences of varied water availibility. *Journal of Ecology* 73: 757–763.

Venable D. L. and D. A. Levin. 1985. Ecology of achene dimorphism in *Heterotheca latifolia*. 1. Achene structure, germination and dispersal. *Journal of Ecology* 73: 133–145.

Venable D.L., Búrquez A., Corral G., Morales E., Esponosa F. 1987. The Ecology of seed heteromorphism in *Heterosperma Pinnatum* in central Mexico. Ecology 68: 65-76

Kigel J. 1992. Diaspore heteromorphism and germination in populations of the ephemeral *Hedypnois rhagadioloides* (L.) F. W. Schmidt (Asteraceae) inhabiting a geographic range of increasing aridity. *Acta Oecologica* 13: 45–53.

Imbert E. 2002. Ecological consequences and ontogeny of seed heteromorphism. *Perspectives in Plant Ecology, Evolution, and Systematics* 5: 13–36.

Washitani, I. 1987. A convenient screening test system and a model for thermal germination responses of wild plant seeds: behavior of model and real seed in the system. *Plant Cell Environ.* 10:587-598.

Chapter 2: Tables

Table 1. Geographical description of sampled *Grindelia ciliata* (Asteraceae) populations

Population	Latitude	Longitude	Climate division
P1	36.9066	-100.5122	Panhandle
P2	36.8108	-99.8919	Panhandle
NC1	36.7979	-98.9357	North-central
NC2	36.8111	-98.0326	North-central
NC3	36.7626	-96.8111	North-central

Chapter 2: Figures

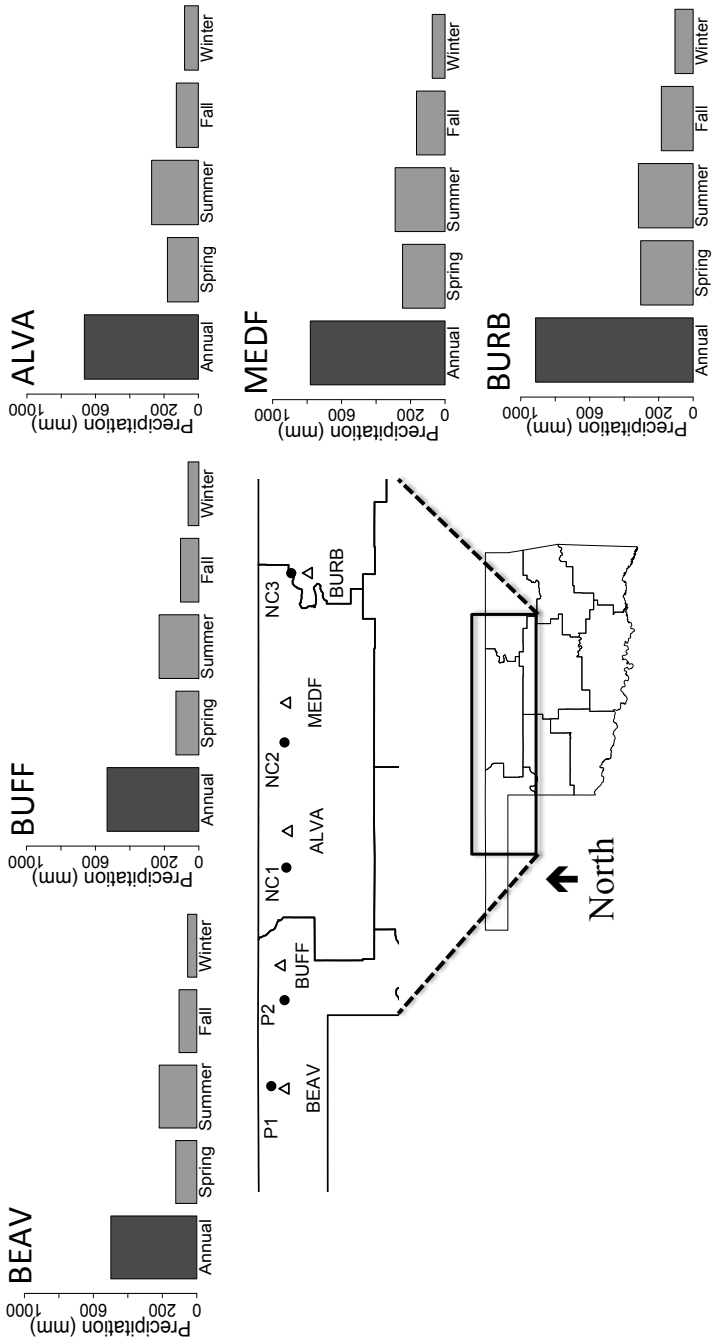

Figure 1. Locations of *Grindelia ciliata* populations (filled circles) sampled for achene dormancy. Open triangles indicate the nearest location of Oklahoma Mesonet stations. Mean Annual, spring, summer, fall, and winter precipitation between 2001-2015 is shown.

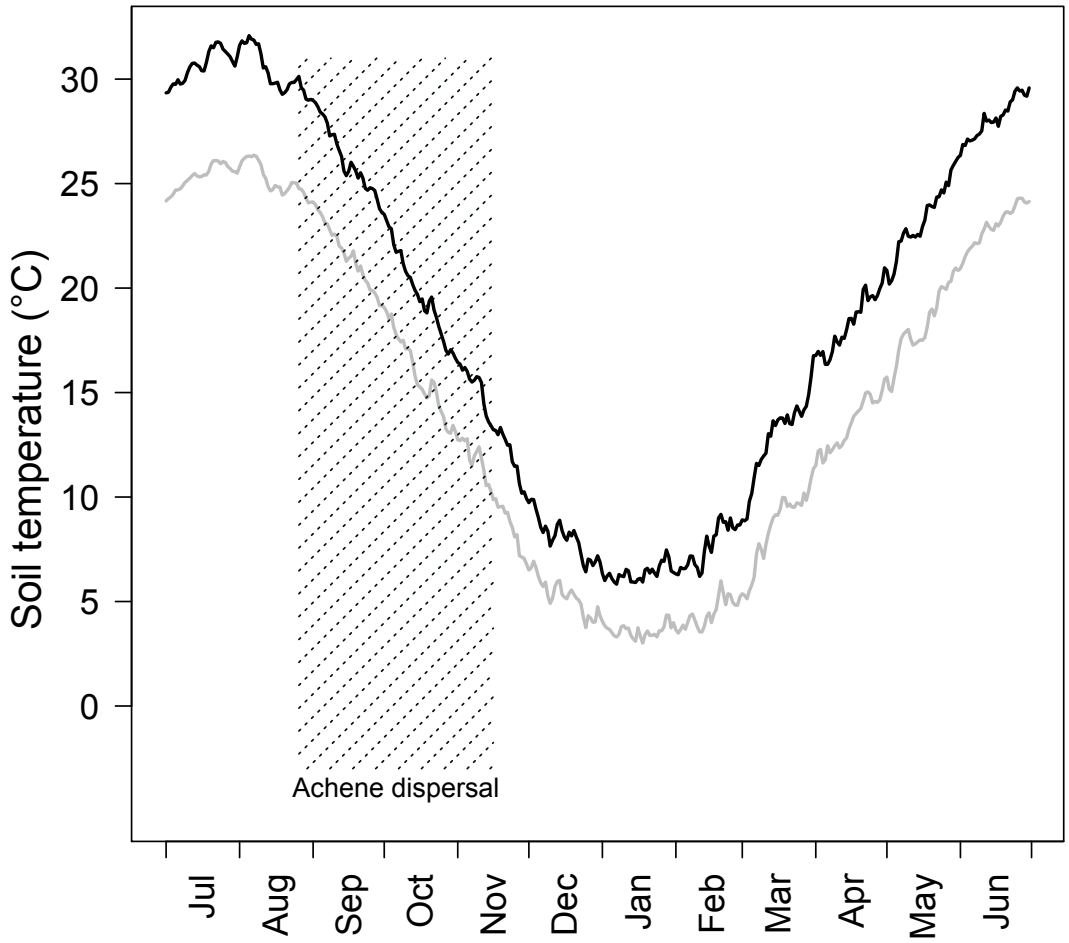

Figure 2. Average annual daily maximum and minimum Oklahoma soil temperatures (solid lines, McPherson et al 2007, Brock et al 1995). Also shown are dispersal timings of *Grindelia ciliata* (Asteraceae) achenes (dashed lines).

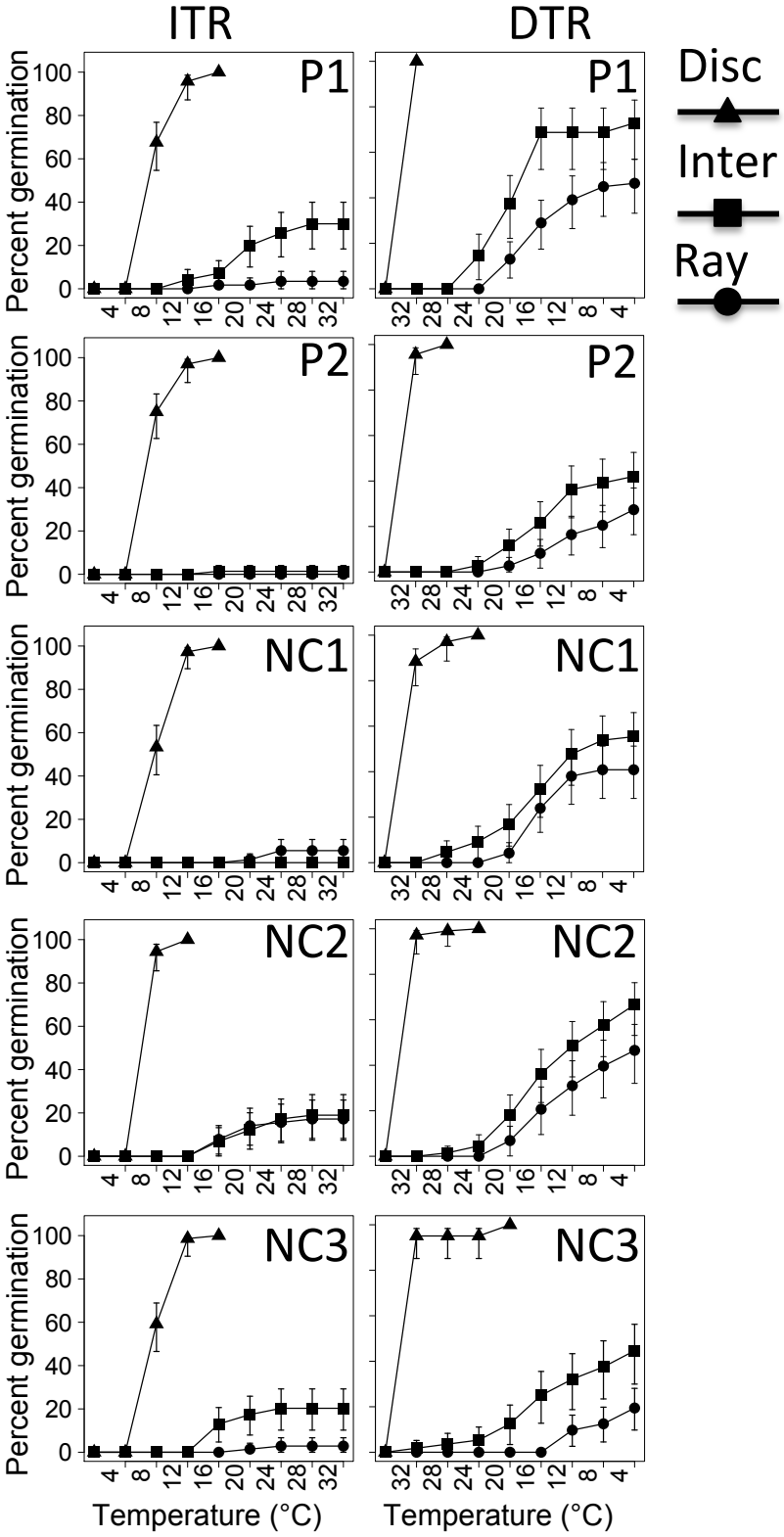
ITR
DTR
Disc
Inter
Ray
P1
P2
NC1
NC2
NC3
Percent germination
Temperature (°C)
0
20
40
60
80
100
4
8
12
16
20
24
28
32

Figure 3. Mean percent germination of fresh *Grindelia ciliata* disc (triangle), intermediate (square), and ray (circle) achenes collected from 5 populations during increasing (ITR, left column) and decreasing (DTR, right column) temperature regimes, during which temperatures were changed by 4°C every 4 days. Error bars indicate 95% confidence intervals. All ungerminated achenes at the end of the germination trial were viable.

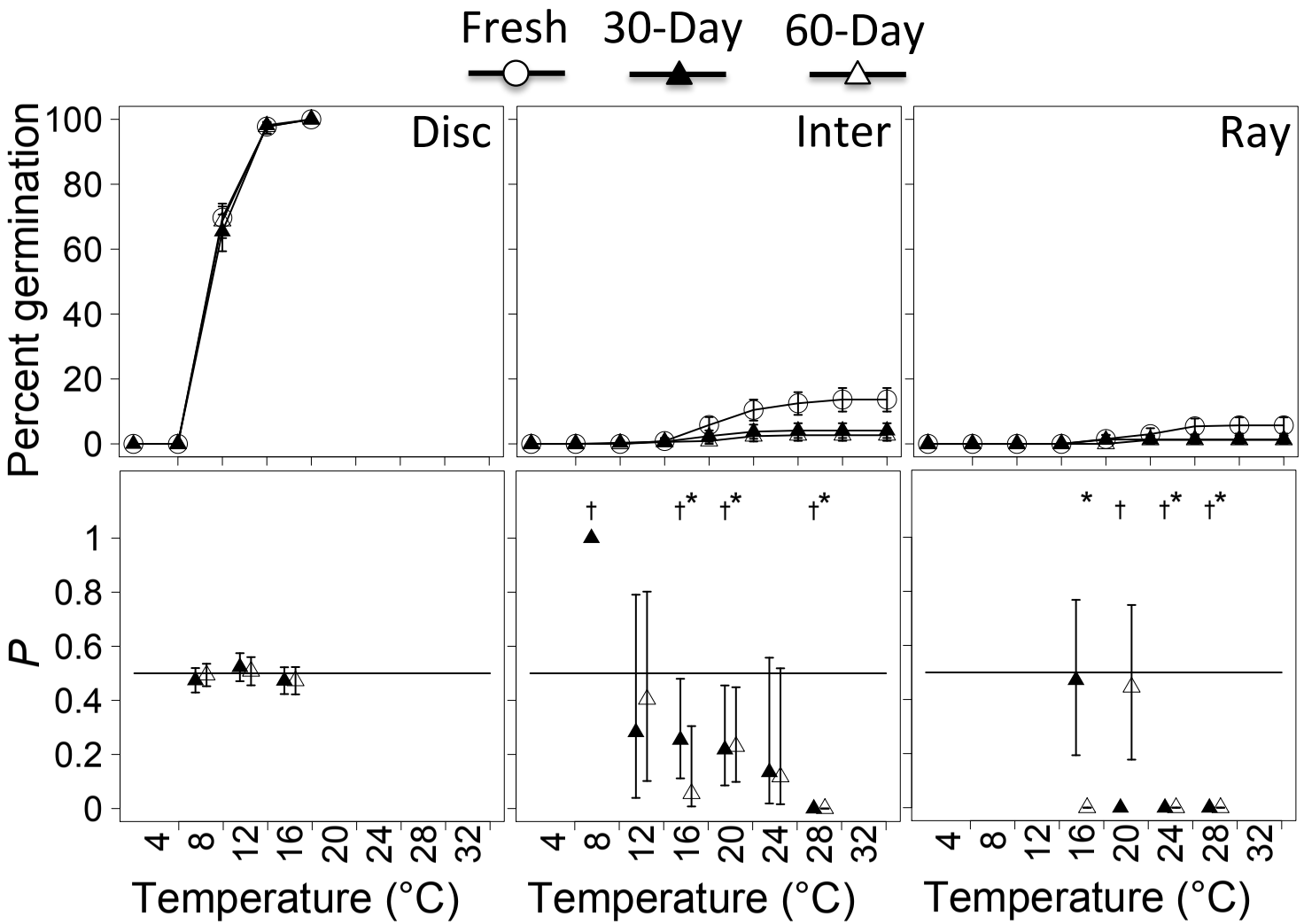
Fresh
30-Day
60-Day
Disc
Inter
Ray
Percent germination
100
80
60
40
20
0
P
1
0.8
0.6
0.4
0.2
0
†
†*
†*
†*
*
†
†*
†*
4
8
12
16
20
24
28
32
Temperature (°C)
Temperature (°C)
Temperature (°C)

Figure 4. Top row shows mean percent germination of fresh (open circle), 30-day CDAR (filled triangle) and 60-day CDAR (open triangle) *Grindelia ciliata* disc, intermediate (inter), and ray achenes during increasing temperature regimes. Bottom row shows mean conditional probability of germination at each temperature relative to fresh achenes. Mean germination probability of 0.5 (solid line) indicates equal probability of germinating for fresh and stored achenes. Probability values above 0.5 indicate a higher probability of germination in stored achenes. Statistical significance ($p \leq 0.05$) is indicated by daggers (†) for 30-day stored achenes, and by double daggers (*) for 60-day stored achenes. Error bars indicate 95% confidence intervals. All ungerminated achenes at the end of the germination trial were viable.

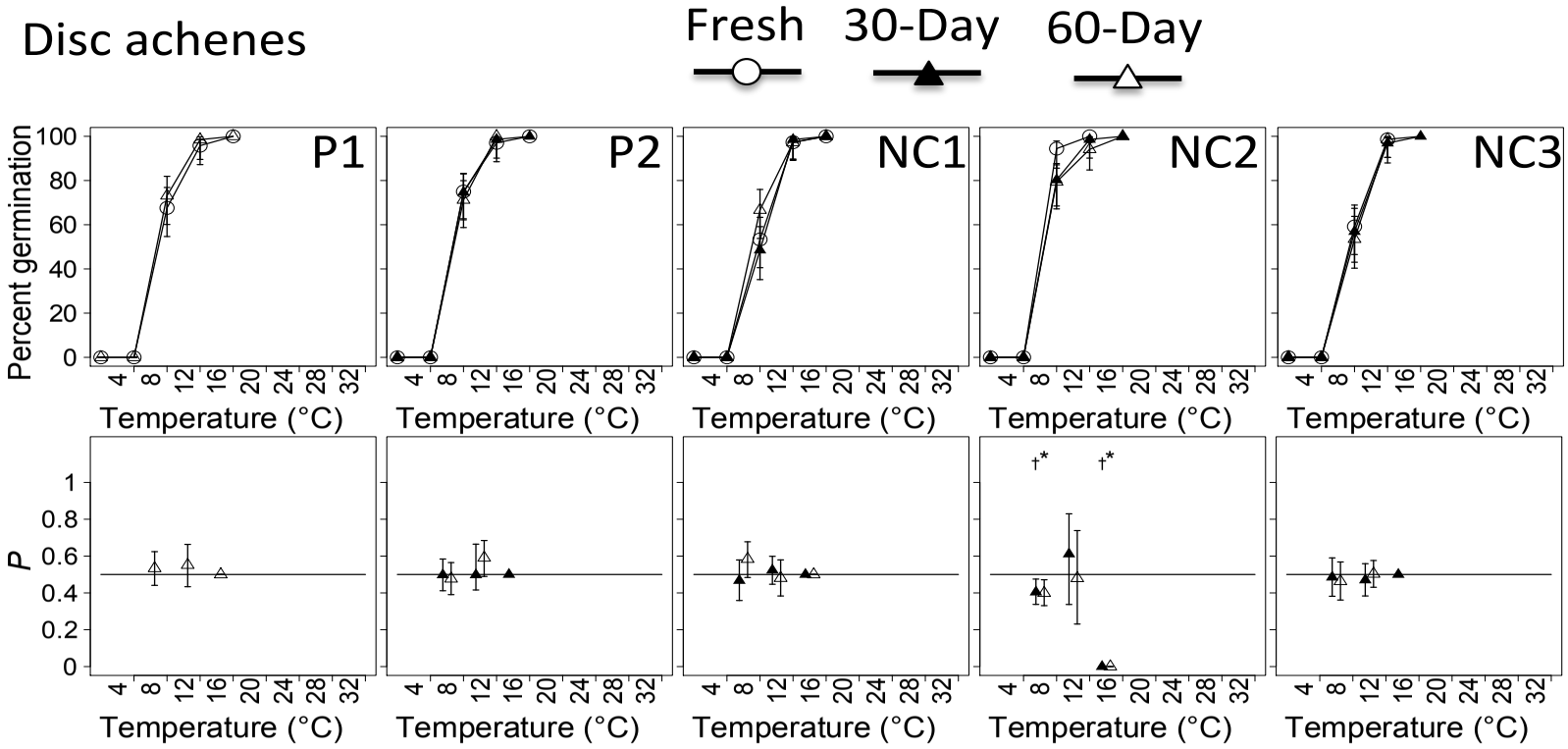
Disc achenes
Fresh
30-Day
60-Day
P1
P2
NC1
NC2
NC3
Percent germination
P
Temperature (°C)
†*

Figure 5. Top row shows mean percent germination of fresh (open circle), 30-day CDAR (filled triangle) and 60-day CDAR (open triangle) *Grindelia ciliata* disc achenes during increasing temperature regimes. Bottom row shows mean conditional probability of germination at each temperature relative to fresh achenes. Mean germination probability of 0.5 (solid line) indicates equal probability of germinating for fresh and stored achenes. Probability values above 0.5 indicate a higher probability of germination in stored achenes. Statistical significance ($p \leq 0.05$) is indicated by daggers (†) for 30-day stored achenes, and by double daggers (*) for 60-day stored achenes. Error bars indicate 95% confidence intervals. All ungerminated achenes at the end of the germination trial were viable.

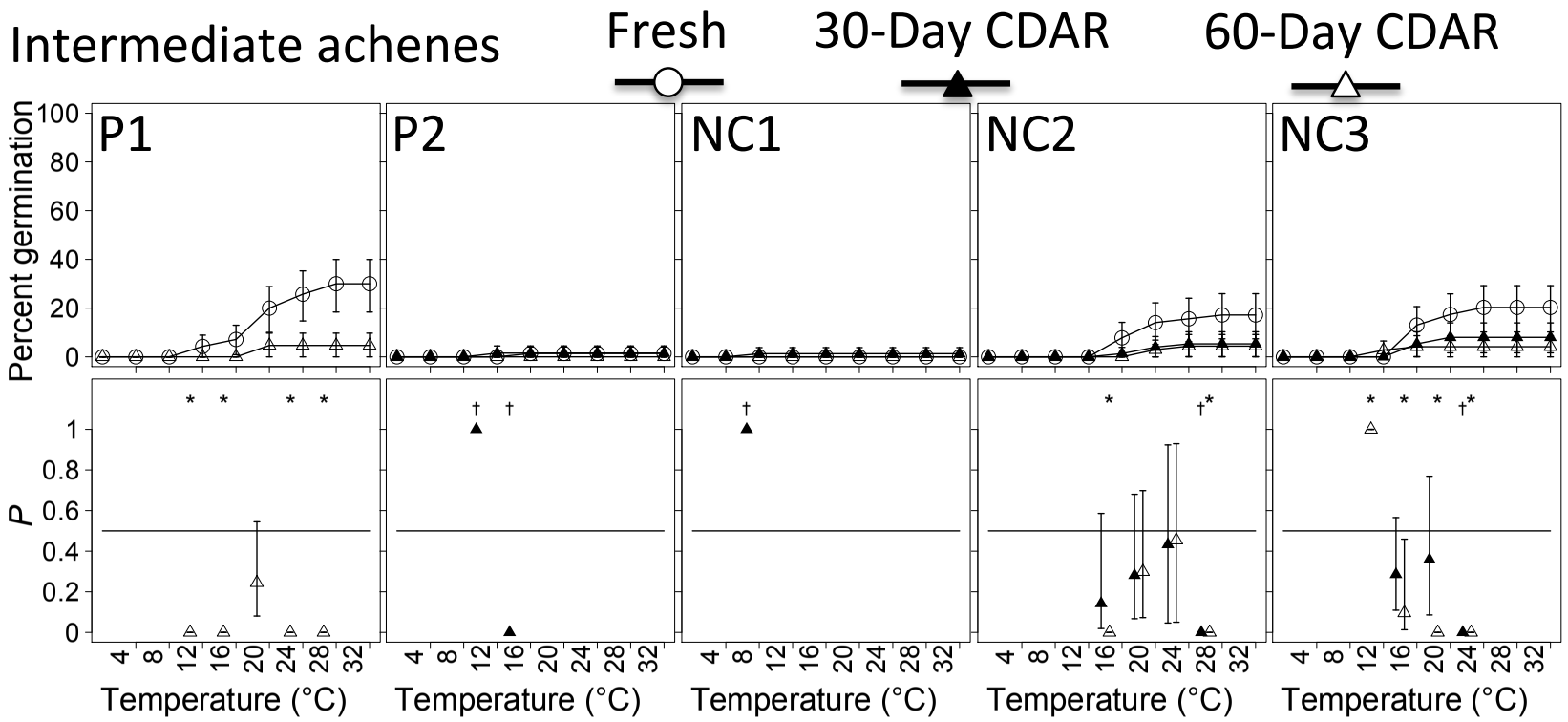
Intermediate achenes
Fresh
30-Day CDAR
60-Day CDAR
P1
P2
NC1
NC2
NC3
Percent germination
P
Temperature (°C)

Figure 6. Mean percent germination of fresh (open circle), 30-day CDAR (filled triangle) and 60-day CDAR (open triangle) *Grindelia ciliata* intermediate achenes during increasing temperature regimes. Bottom row shows mean conditional probability of germination at each temperature relative to fresh achenes. Mean germination probability of 0.5 (solid line) indicates equal probability of germinating for fresh and stored achenes. Probability values above 0.5 indicate a higher probability of germination in stored achenes. Statistical significance ($p \leq 0.05$) is indicated by daggers (†) for 30-day stored achenes, and by double daggers (*) for 60-day stored achenes. Error bars indicate 95% confidence intervals. All ungerminated achenes at the end of the germination trial were viable.

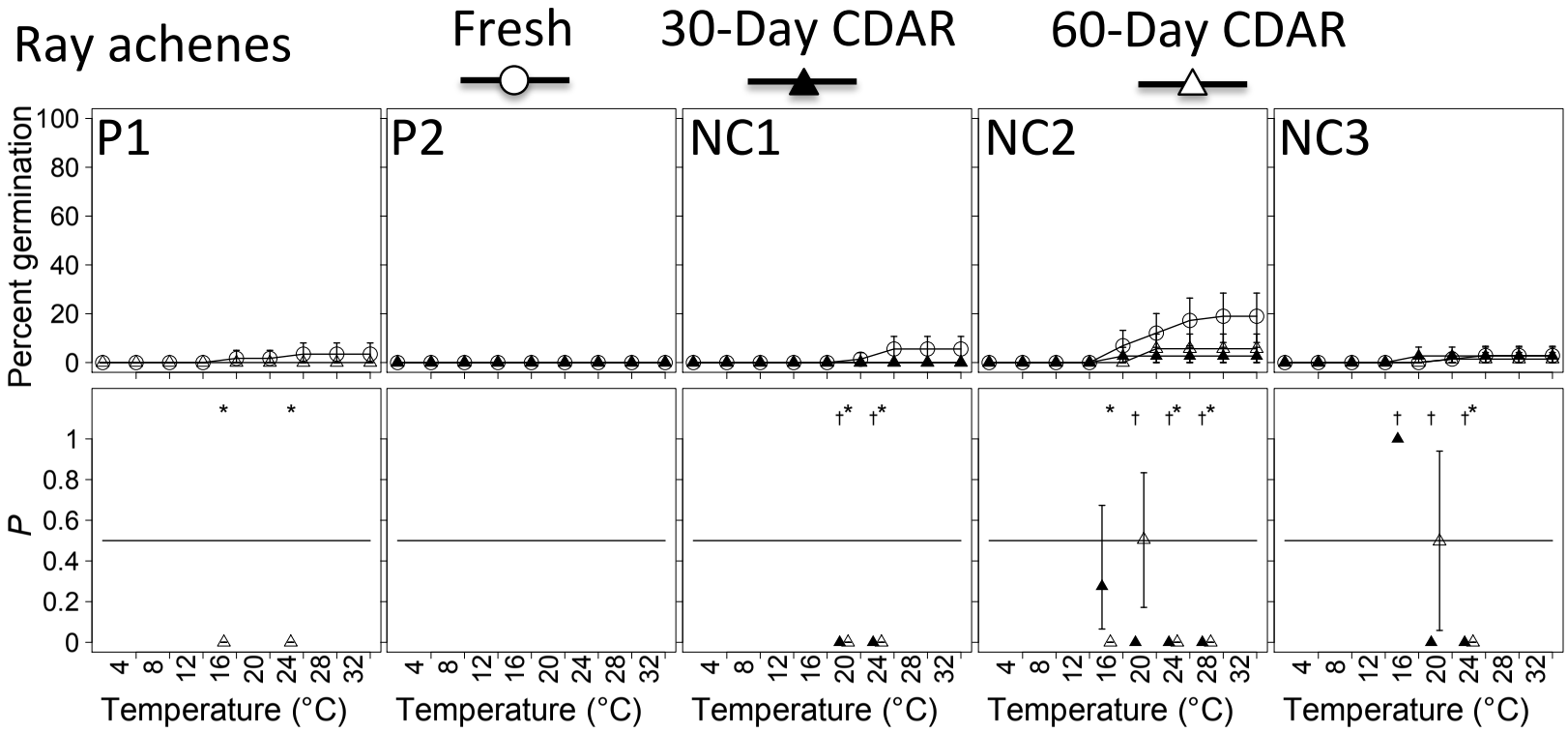
Ray achenes
Fresh
30-Day CDAR
60-Day CDAR
P1
P2
NC1
NC2
NC3
Percent germination
P
Temperature (°C)

Figure 7. Mean percent germination of fresh (open circle), 30-day CDAR (filled triangle) and 60-day CDAR (open triangle) *Grindelia ciliata* ray achenes during increasing temperature regimes. Bottom row shows mean conditional probability of germination at each temperature relative to fresh achenes. Mean germination probability of 0.5 (solid line) indicates equal probability of germinating for fresh and stored achenes. Probability values above 0.5 indicate a higher probability of germination in stored achenes. Statistical significance ($p \leq 0.05$) is indicated by daggers (†) for 30-day stored achenes, and by double daggers (*) for 60-day stored achenes. Error bars indicate 95% confidence intervals. All ungerminated achenes at the end of the germination trial were viable.

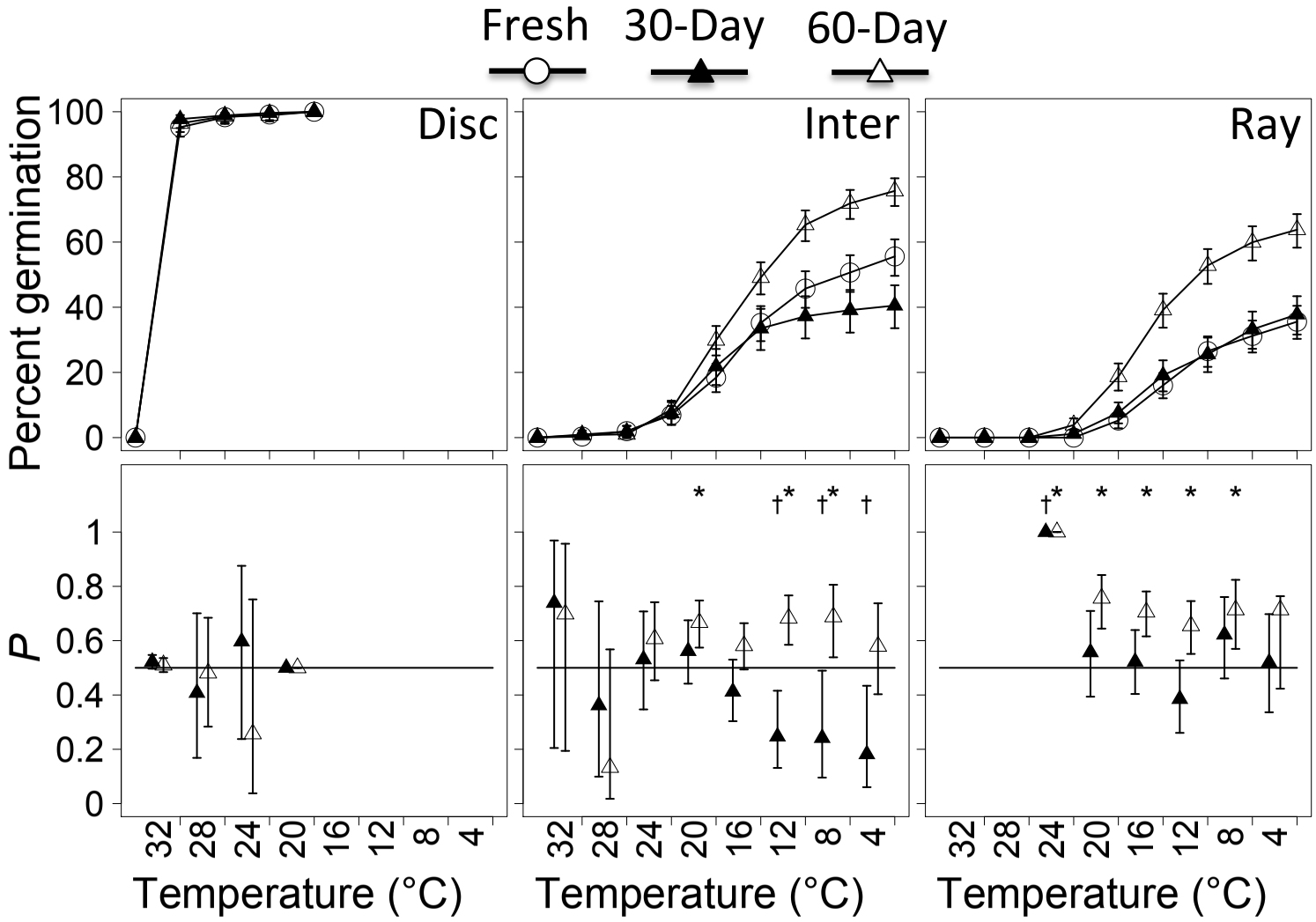
Fresh
30-Day
60-Day
Disc
Inter
Ray
Percent germination
100
80
60
40
20
0
P
1
0.8
0.6
0.4
0.2
0
32
28
24
20
16
12
8
4
Temperature (°C)
Temperature (°C)
Temperature (°C)
*
†*
†*
†
†*
*
*
*
*

Figure 8. Top row shows mean percent germination of fresh (open circle), 30-day WDAR (filled triangle) and 60-day WDAR (open triangle) *Grindelia ciliata* disc, intermediate (inter), and ray achenes during decreasing temperature regimes. Bottom row shows mean conditional probability of germination at each temperature relative to fresh achenes. Mean germination probability of 0.5 (solid line) indicates equal probability of germinating for fresh and stored achenes. Probability values above 0.5 indicate a higher probability of germination in stored achenes. Statistical significance ($p \leq 0.05$) is indicated by daggers (†) for 30-day stored achenes, and by double daggers (*) for 60-day stored achenes. Error bars indicate 95% confidence intervals. All ungerminated achenes at the end of the germination trial were viable.

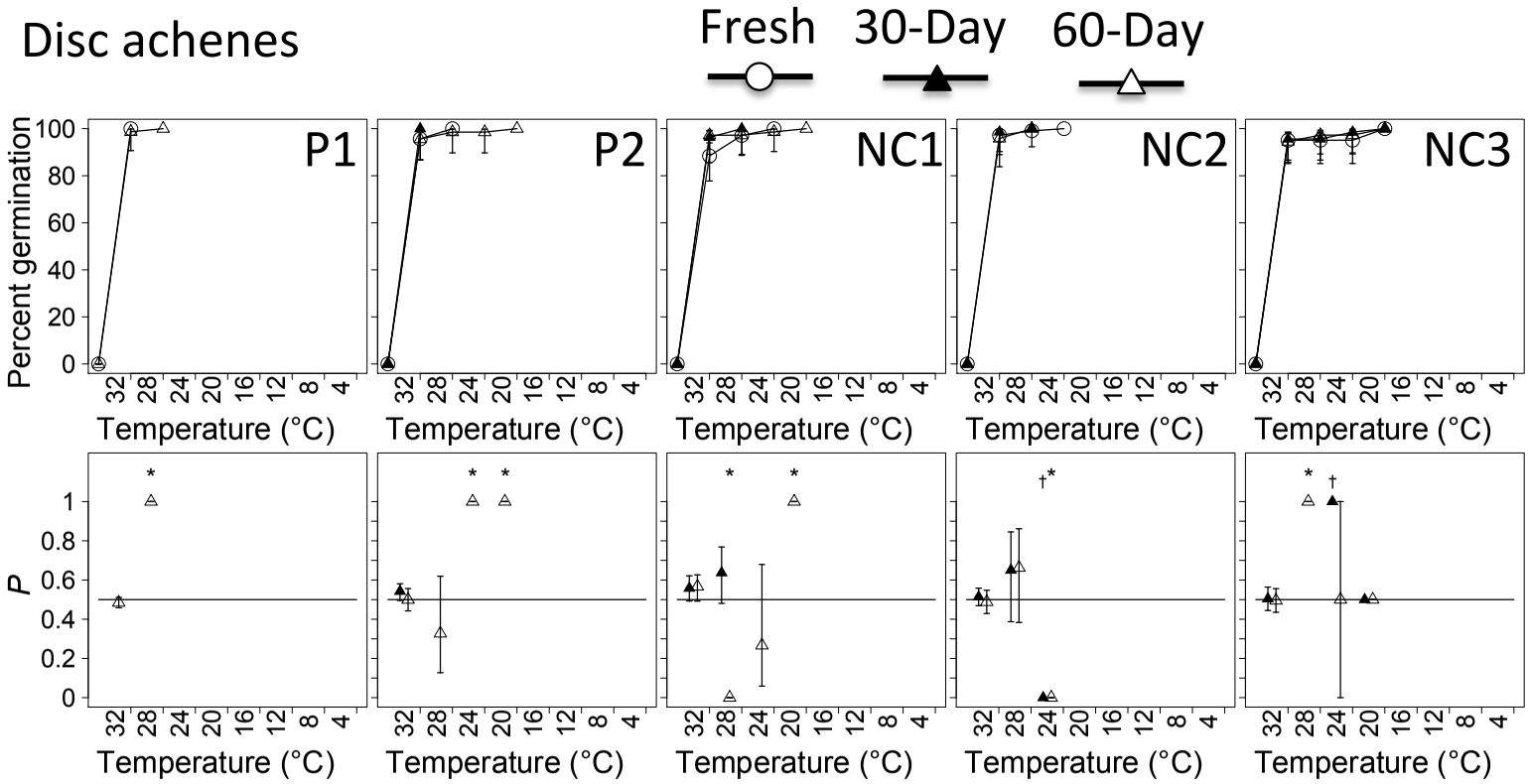
Disc achenes
Fresh
30-Day
60-Day
P1
P2
NC1
NC2
NC3
Percent germination
100
80
60
40
20
0
P
1
0.8
0.6
0.4
0.2
0
32 28 24 20 16 12 8 4
Temperature (°C)
*
†*
†

Figure 9. Top row shows mean percent germination of fresh (open circle), 30-day WDAR (filled triangle) and 60-day WDAR (open triangle) *Grindelia ciliata* disc achenes during decreasing temperature regimes. Bottom row shows mean conditional probability of germination at each temperature relative to fresh achenes. Mean germination probability of 0.5 (solid line) indicates equal probability of germinating for fresh and stored achenes. Probability values above 0.5 indicate a higher probability of germination in stored achenes. Statistical significance ($p \leq 0.05$) is indicated by daggers (†) for 30-day stored achenes, and by double daggers (*) for 60-day stored achenes. Error bars indicate 95% confidence intervals. All ungerminated achenes at the end of the germination trial were viable.

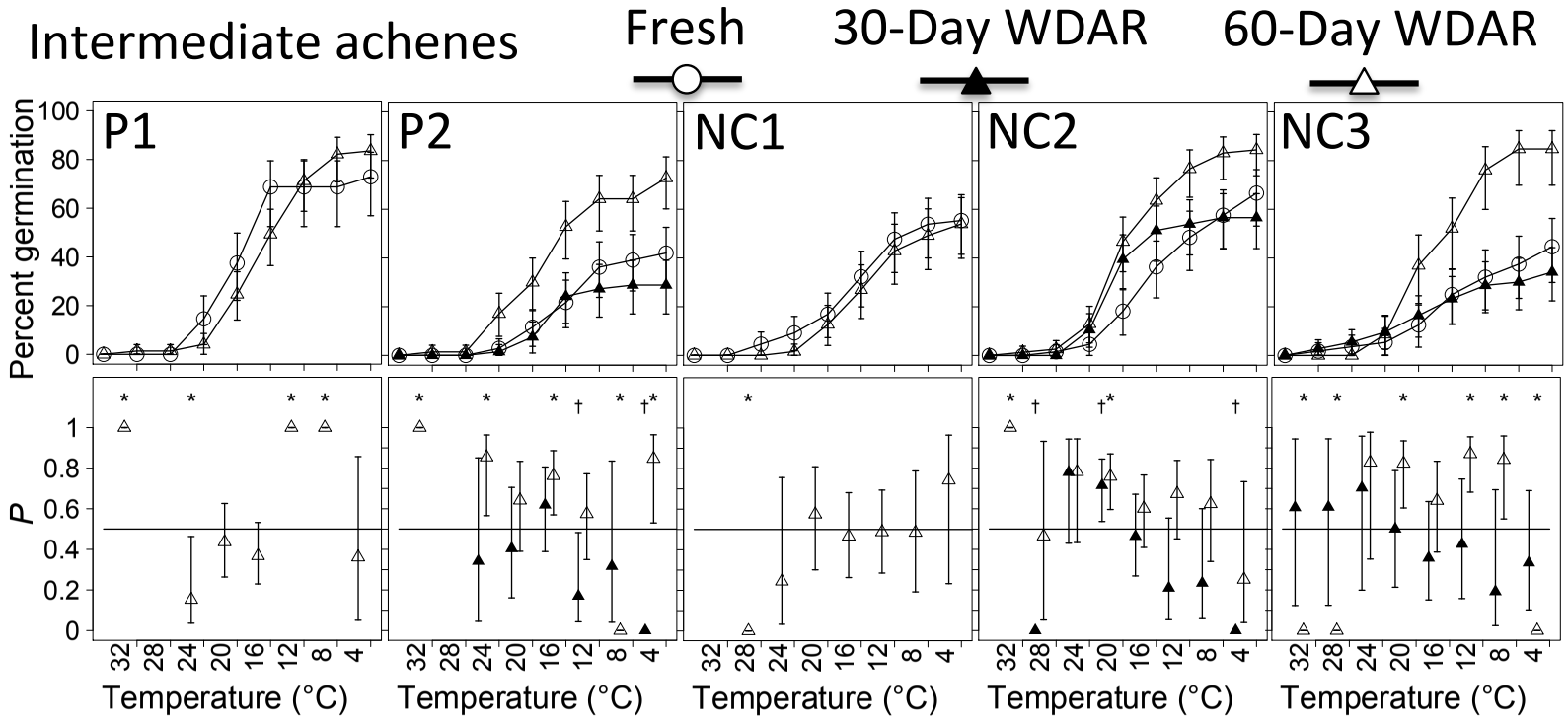
Intermediate achenes
Fresh
30-Day WDAR
60-Day WDAR
P1
P2
NC1
NC2
NC3
Percent germination
P
Temperature (°C)

Figure 10. Mean percent germination of fresh (open circle), 30-day WDAR (filled triangle) and 60-day WDAR (open triangle) *Grindelia ciliata* intermediate achenes during decreasing temperature regimes. Bottom row shows mean conditional probability of germination at each temperature relative to fresh achenes. Mean germination probability of 0.5 (solid line) indicates equal probability of germinating for fresh and stored achenes. Probability values above 0.5 indicate a higher probability of germination in stored achenes. Statistical significance ($p \leq 0.05$) is indicated by daggers (†) for 30-day stored achenes, and by double daggers (*) for 60-day stored achenes. Error bars indicate 95% confidence intervals. All ungerminated achenes at the end of the germination trial were viable.

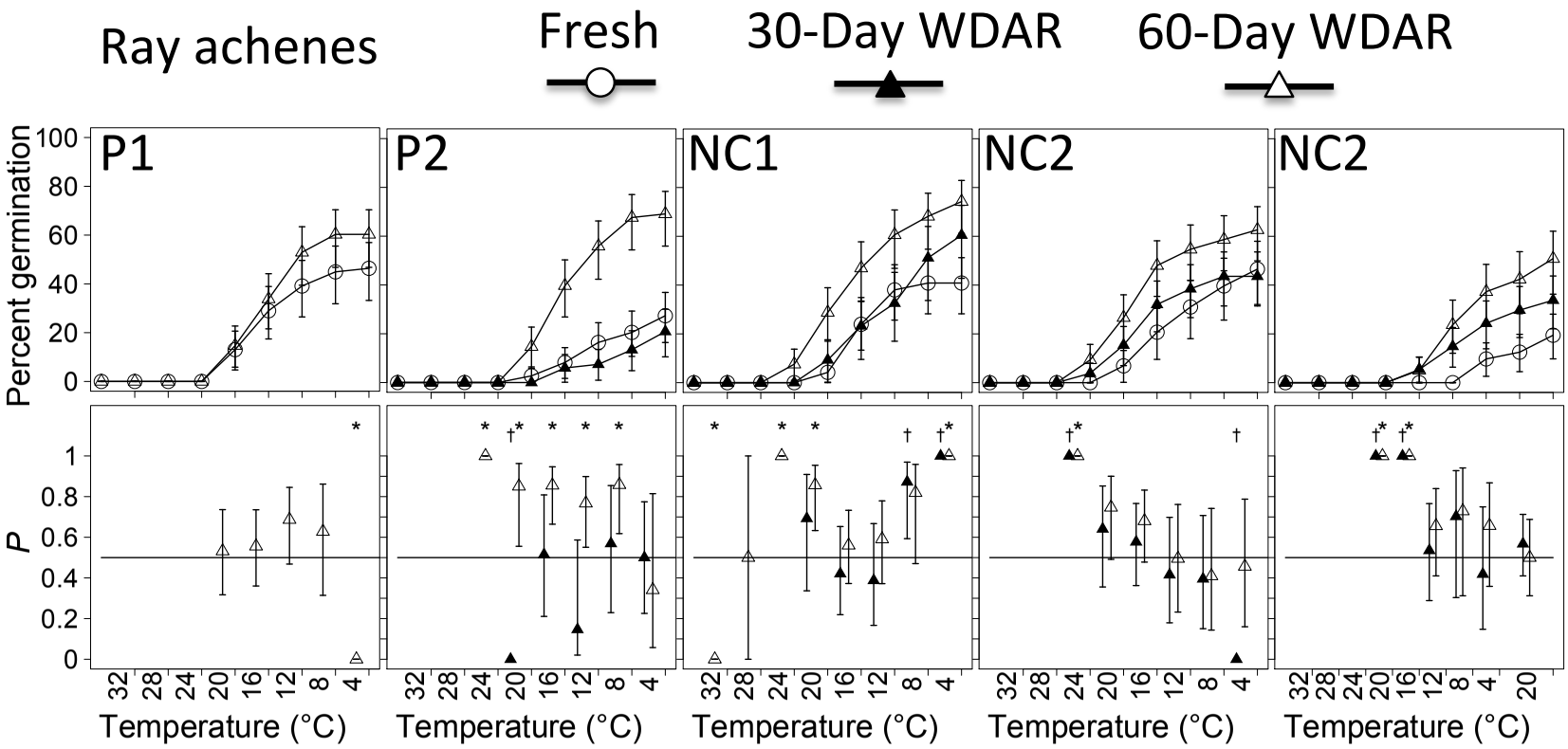
Ray achenes
Fresh
30-Day WDAR
60-Day WDAR
P1
P2
NC1
NC2
NC2
Percent germination
P
Temperature (°C)

Figure 11. Mean percent germination of fresh (open circle), 30-day WDAR (filled triangle) and 60-day WDAR (open triangle) *Grindelia ciliata* ray achenes during decreasing temperature regimes. Bottom row shows mean conditional probability of germination at each temperature relative to fresh achenes. Mean germination probability of 0.5 (solid line) indicates equal probability of germinating for fresh and stored achenes. Probability values above 0.5 indicate a higher probability of germination in stored achenes. Statistical significance ($p \leq 0.05$) is indicated by daggers (†) for 30-day stored achenes, and by double daggers (*) for 60-day stored achenes. Error bars indicate 95% confidence intervals. All ungerminated achenes at the end of the germination trial were viable.

Chapter 3: Bet-hedging against larval herbivory and seed bank mortality in the evolution of heterocarpy

Michael Kistenmacher, University of Oklahoma, Department of Microbiology and Plant Biology 770 Van Vleet Oval, Norman, Oklahoma 73019 USA

J. Phil Gibson, University of Oklahoma, Department of Biology, 730 Van Vleet Oval, Norman, Oklahoma 73019 USA

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Keywords: Asteraceae; bet-hedging; capitula; evolutionarily stable strategies; *Grindelia*, heterocarpy; net reproductive rate; Noctuidae; plant–insect interaction; seed bank

Chapter 3: Abstract

PREMISE OF THE STUDY— Bet-hedging strategies maximize long-term geometric fitness at the cost of reduced arithmetic fitness by offsetting different mortality risks. Heterocarpic systems accomplish bet-hedging through the production of two or more fruit types that vary in dormancy and dispersal ability. It is unknown whether heterocarpy also offsets predispersal mortality risks. To

address this question, we investigated whether heterocarpy in *Grindelia ciliata* (Asteraceae) also offsets mortality risks posed by a seed predator *Schinia mortua* (Noctuidae) to increase plant fitness.

METHODS—We conducted two manipulative experiments to quantify critical life history components of this plant–insect interaction. We measured predispersal achene mortality from herbivory, postdispersal achene mortality in the seed bank, and seedling emergence. These measurements were then used in deterministic models to evaluate evolutionary consequences of predispersal seed mortality in *G. ciliata*.

KEY RESULTS—Dormant achene types were less vulnerable to herbivory but more susceptible to mortality in the seed bank due to delayed seed emergence. Nondormant achene types experienced high predispersal mortality but low seed bank mortality due to rapid germination. Our herbivore-dependent model improved fit between observed and expected proportions of dormant and nondormant *G. ciliata* achenes and showed that heterocarpy could evolve in the absence of postgermination mortality.

CONCLUSIONS—Our study provides empirical support of how predispersal herbivory can be equally important to postdispersal seed mortality risks in the evolution and maintenance of a heterocarpic reproductive system and expands understanding of how bet-hedging theory can be used to understand this unique reproductive strategy.

Chapter 3: Introduction

A universal risk for plants is the possibility that seedlings will not survive to reproduce. Bet-hedging reproductive strategies can increase the likelihood of successfully producing offspring in environments where favorable conditions for establishment and survival are temporally or spatially variable because, although arithmetic mean fitness is lower, they increase geometric mean fitness by spreading mortality risks among dissimilar offspring, thereby reducing variation in fitness among years (Cohen, 1966; Gillespie, 1974; Seger and Brockmann, 1987; Roff, 2002).

Heterocarpy is a reproductive bet-hedging strategy in which plants produce two or more types of fruits that are ecologically distinct from one another. Because of their differences, seeds have dissimilar dispersal and germination responses, which allows them to increase the probability of establishing seedlings despite spatial and temporal variation in "good" and "bad" conditions. Most studies have evaluated bet-hedging through heterocarpy in arid environments where bad years refer to those with high drought-induced seedling mortality (Venable, 1985a; Venable and Levin, 1985; Brown and Venable, 1986; Venable *et al.*, 1987; Philippi, 1993a; Clauss and Venable, 2000; reviewed by Evans and Dennehy, 2005). However, other postdispersal mortality factors, such as sibling competition, can also shape evolution of heterocarpic systems (Ellner, 1986; Ellner, 1987). What has not been explored is whether bet-hedging is also adaptive for offsetting predispersal seed mortality risks. In this study, we tested Imbert's (2002) idea that predispersal herbivory can pose a significant risk with the capacity to drive the evolution of a bet-hedging strategy.

There are two types of bet-hedging strategies. A conservative bet-hedging strategy (i.e., "a jack of all trades but master of none") uses one generalist phenotype that is equally good in favorable and unfavorable conditions. In contrast, a diversified bet-hedging strategy (i.e., "Don't put all your eggs in one basket.") produces multiple distinct phenotypes that are specialists for different conditions (Cohen, 1966; Slatkin, 1974; Cooper and Kaplan, 1982; Seger and Brockmann, 1987; Philippi and Seger, 1989; Starrfelt and Kokko, 2012). Our study focuses on the diversified bet-hedging strategies of heterocarpy, in which resources are allocated among multiple morphologically and ecologically distinct fruits (Harper, 1977; Philippi, 1993b; de Clavijo, 1994; Imbert, 2002; Mandák and Pyšek, 2001a; Evans and Dennehy, 2005; Crean and Marshall, 2009; Childs *et al.*, 2010). Heterocarpy has evolved independently in multiple plant families, but is particularly common in the Asteraceae (Venable, 1985a, b; Venable *et al.*, 1995; Imbert, 2002). Heterocarpic members of this family typically produce ray and disc florets that develop single-seeded fruits (achenes) that differ in seed size and mass (Ellner and Shmida, 1984; Maxwell *et al.*, 1994), seed and pericarp composition (Venable and Levin, 1985; Gutterman, 1994a; Jaimand and Rezaee, 1996), or the presence or absence of dispersal structures (Baker and O'Dowd, 1982). Morphological differences between achene types can be discrete or show continuous variation in features, resulting in multiple achene types that share different combinations of traits (Zohary, 1950; Pomplitz, 1956; Bachmann *et al.*, 1984).

Venable (1985b) described heterocarpy as a high-risk–low-risk bet-hedging strategy because the dissimilar achene types offset different seed and seedling mortality risks. One achene type represents investment in high-risk propagules that disperse away

from the parental plant and germinate immediately. The alternate achene type reflects investment in low-risk propagules that disperse locally, are dormant, and consequently contributes to a soil seed bank. High-risk achenes are adaptive due to high arithmetic mean fitness experienced through them during favorable years when there is a high probability of postgermination survival because each seed germinates soon after dispersal and establishes a new individual that has the potential to produce many offspring. However, high-risk achenes can experience high seedling mortality due to unpredictability of spatial dispersal or when their lack of dormancy exposes seedlings to unfavorable conditions immediately following germination. Although low-risk achenes have lower arithmetic mean fitness during favorable years (Brown and Venable, 1986; Venable, 1985b), they offset the risks of distant dispersal and immediate germination by remaining dormant in the seed bank around the maternal plant and by delaying germination. However, this strategy comes at the risk of mortality in the seed bank. Producing multiple achene types with dissimilar features may be adaptive in unpredictable environments because the plant is able to produce seeds that can colonize new locations and immediately take advantage of favorable conditions, as well as seeds that can contribute to a local seed bank, germinate at a later time, and consequently minimize the risk of local extinction. In monocarpic plants, this life history strategy substitutes for the adaptive benefits of iteroparity by staggering emergence of offspring produced in one clutch (Murphy, 1968).

Although different aspects of the evolutionary ecology of heterocarpic bet-hedging strategies have been described for a number of species, the optimal ratio(s) of nondormant to dormant achenes has not been resolved. Determining a single optimal

ratio for particular environments is challenging because mortality risks vary at different life stages and can be affected by a variety of stochastic biotic (i.e., competition, herbivory) or abiotic (i.e., water availability, fire) factors that may occur unpredictably. The delayed germination model of Cohen (1966) predicts that the ratio of dormant to nondormant seeds is directly related to the probability of experiencing years with high seedling mortality (i.e., bad years). In this model, allocation strategies with large proportions of dormant seeds are correlated with environments that experience a high probability of bad years, whereas allocation strategies with large proportions of nondormant seeds are correlated with environments that experience a low probability of bad years (Childs *et al.*, 2010). Most empirical evaluations of bet-hedging theory related the probability of mortality in the seed bank or drought-induced seedling mortality with optimal achene allocations strategies. However, other sources of mortality such as predispersal seed predation could be equally important (Imbert, 2002). Predispersal seed predation influences the evolution of a variety of plant traits (Kolb *et al.*, 2007, Fenner *et al.* 2002), causes severe seed loss (Salisbury, 1942; Janzen, 1971; Louda, 1978, 1982b), and limits plant recruitment, abundance, and distribution (Huffaker and Kennett, 1959; Harper, 1969; Goeden and Ricker, 1977; Goeden and Ricker, 1978; Louda, 1982b).

To study the evolutionary consequences of predispersal herbivory in a heterocarpic system, we included predispersal seed predation in a bet-hedging model (Cohen, 1966). We derived pre- and postdispersal mortality values used in this model from field measurements of plants of *Grindelia ciliata* (Asteraceae, Spanish gold, wax goldenweed) with achenes that have been subject to herbivory by *Schinia mortua*

(Noctuidae) larvae. This naturally occurring plant–insect system provides an exceptional case for studying the influence of predispersal mortality risks on heterocarpy. *Grindelia ciliata* produces disc, intermediate, and ray achenes. Disc achenes germinate quickly, have a thin pericarp, large seed, and retain a functional pappus (Fig. 1), whereas ray and intermediate achene germination is delayed (Gibson, 2001), have a thick lignified pericarp, and a small seed. Intermediate achenes retain a functional dispersal pappus, whereas ray achenes do not (Fig. 1). *Schinia mortua* females oviposit into *G. ciliata* capitula and hatched larvae feed on immature and mature achenes (J. P. Gibson). Much of the life histories of *G. ciliata* and *S. mortua* are unknown. Therefore, we gather basic information about their life history stages and interactions through two experiments.

In the first experiment, we measured predispersal risks by studying the consequences of *S. mortua* oviposition selectivity, which is commonly debated as a mechanism for plant–insect coevolution, sympatric speciation, and patterns of attack on host plants (Thompson and Pellmyr, 1991). We specifically tested how the timing of larval hatching in *G. ciliata* capitula affects both larval and achene mortality. We predicted that heterocarpy in *G. ciliata* will offset predispersal achene mortality caused by larval herbivory because the thick, lignified pericarp of intermediate and ray achenes should provide protection from consumption by *S. mortua* larvae. The thin pericarp of disc achenes should provide less protection and render them more susceptible to consumption by *S. mortua* larvae. However, we expected the susceptibility of each achene type and, consequently, larval survival, to vary depending on the synchronization between larval and achene development. A mismatch in

synchronization (e.g., early-instar larvae attempting to feed on achenes in the final stages of development) may result in high larval but low achene mortality due to achene pericarps beginning to lignify and harden. In contrast, early instars feeding on young achenes could increase both larval survival and achene mortality due to the pericarps not yet being lignified and, therefore, being vulnerable to feeding larvae.

Our second experiment focused on determining postdispersal achene mortality risks. We measured seedling emergence, achene mortality, and achene longevity in the seed bank to evaluate how heterocarpy in *G. ciliata* could offset these risks. Nondormant disc achenes should germinate immediately, offsetting mortality risks faced by dormant seeds in the seed bank. In contrast, dormant achenes should contribute to a persistent soil seed bank, which offsets the risks of immediate germination following dispersal. Because we did not know what conditions break dormancy or when seedlings naturally emerge from the seed bank, we tested two common dormancy-breaking mechanisms, stratification and scarification, to determine whether winter soil temperatures or physical abrasion of the pericarp could reduce dormancy and lead to spring seedling emergence.

In the final component of the study, we used data from the previous two experiments to model the evolutionary consequences of predispersal mortality in this bet-hedging system. We combine the results using the case of two outcomes model (Cohen, 1966) to simulate theoretical environments that vary in both the frequency of good and bad years (i.e., drought and nondrought years) and the intensity of herbivory to predict the ratio of achene types that maximize geometric mean fitness. We compared those predictions to achene ratios observed in field and greenhouse plants to evaluate

the role of predispersal mortality in this species. These studies not only provide insights on the *Grindelia–Schinia* system but also investigated aspects of plant reproductive bet-hedging theory that have not been fully explored.

Chapter 3: Materials and Methods

Study species—

Grindelia ciliata (Nutt.) Nutt. (Astereae, Asteraceae) is a widely distributed short-lived herbaceous annual native to the southwestern United States and northward through the Great Plains (Steyermark, 1934). It typically grows along roadsides, agricultural areas, and disturbed sites. Individuals are monocarpic, and juveniles grow as a rosette that bolts between May and June to form a single stem or multiple stems that will each typically produce one to six capitula that flower between August and October. Capitula contain ray and disc florets (Fig. 1). Ray florets are pistillate and produce ray achenes (Fig. 1) that are small (ca. 2 mm long), epappose, glabrous, slightly globose, and have a thick, lignified pericarp (Gibson, 2001). Disc florets are hermaphroditic and produce intermediate or disc achenes (Fig. 1) from peripherally and centrally located disc florets, respectively (Gibson, 2001). Intermediate achenes are similar to ray achenes in size, shape, and pericarp thickness, but similar to disc achenes in that they produce a prominent pappus of stiff capillary bristles (Fig. 1). Disc achenes are glabrous, almost twice as long (4 mm) as ray and intermediate achenes, have a thin pericarp, and produce a pappus of stiff capillary bristles (Gibson, 2001, Fig. 1). Here we use the word achene to refer to the collective dispersal unit of the seed and pericarp.

Ray and intermediate achene dormancy is physically imposed by the pericarp, as in *Grindelia squarrosa* (McDonough, 1975).

Schinia mortua (Lepidoptera, Noctuidae, Heliothentinae) is sympatric with *G. ciliata* in the southern Great Plains of North America (Grote, 1874; Lafontaine and Schmidt, 2010). Females of various *Schinia* species that use Asteraceae hosts oviposit between disc florets. Larvae (caterpillars) hatch and consume achenes as they mature and then metamorphose into pupa that overwinter in the soil (Hardwick, 1971; Zwick and Estes, 1981; Byers, 1989).

Predispersal seed mortality—

To determine achene mortality risks from *S. mortua* larvae, we grew a cohort of *G. ciliata* plants and exposed their capitula to *S. mortua* larvae. In January 2013, disc achenes were chosen from a pool of achenes collected from individuals grown at the University of Oklahoma Kessler Atmospheric and Ecological Research Station (KAEFS, 34.98549, −97.52351), McClain County, Oklahoma, USA. All plants for this experiment were grown from those achenes. The seedlings germinated in January and therefore were a few months behind in development as compared with seedlings germinated in nature. To synchronize development of our seedlings with natural seedlings, we grew our seedlings under a long day (16 h light/8 h dark) cycle in a growth chamber between January and April 2013. In April 2013, individual rosettes were transplanted into 3.78 L pots filled with Metro Mix Professional Growing Mix 300 (Sun Gro, Agawam, Massachusetts, USA) and placed in a greenhouse exposed to natural light. Plants were watered daily and fertilized once per week with Jack's Professional (20–20–20) Balanced Water Soluble Fertilizer (JR Peters, Allentown,

Pennsylvania, USA). Upon bolting in June 2013, plants were transported to KAEFS. Groups of six plants were placed into a Nesting Tote Box (51.4 × 38.7 × 12.7 cm [length × width × height], Global Equipment, Port Washington, New York, USA) in which a water level of 1–3 cm was maintained throughout the experiment. Nesting totes were placed inside a mesh cage (1.83 × 1.83 × 1.83 m, 12.6 × 12.6 threads/cm Mesh Lumite; Bioquip, Rancho Dominguez, California [CA], USA) exposed to natural light for the remainder of the experiment.

Schinia mortua eggs were collected between August and September 2013 from capitula on wild plants in a population at KAEFS. However, since the date-of-hatching was unknown, eggs were incubated in a laboratory, and hatched larvae were transferred into *G. ciliata* capitula. Eggs were placed in a Petri dish containing one piece of Whatman #1 9-cm filter paper. Dishes were placed into a Precision Model 815 Low Temperature Illuminated Incubator (Pacific Combustion Engineering Co., Torrance, CA, USA) at a constant 22°C and a 12 h/12 h light/dark cycle, which is similar to the average daily temperature and light/dark cycles experienced by eggs in the field at KAEFS. Dishes were checked multiple times a day for hatched larvae. Hatching was scored when the eggshell was eaten and the larval head was protruding from the shell. Hatched larvae were transported to KAEFS in a separate Petri dish lined with one Whatman #1 filter paper. All larvae were moved to and from Petri dishes by an aspirator made of 0.64 cm polyurethane tubing, a 1000 µL pipette tip and a laboratory wipe (Kimwipe, Kimberly Clark) inserted into the pipette tip.

To investigate the influence of oviposition timing on achene mortality, we inoculated the primary apical capitula of 77 plants at specific developmental stages with

a newly hatched first instar larva. We placed larvae into capitula at the 1st (D_1, $n = 15$), 4th (D_4, $n = 15$), 7th (D_7, $n = 17$), 14th (D_{14}, $n = 15$), or 21st (D_{21}, $n = 15$) day of capitulum opening. Newly hatched caterpillars were placed head-first into capitula between centrally located florets using an aspirator. All capitula were assigned treatments on their first day of flowering by drawing a number (1–5, each referring to a specific treatment) out of a hat. Pollen was collected from wild plants daily and was applied with a paintbrush to flowering capitula.

Statistical analysis of predispersal seed mortality—

Once matured, capitula were harvested and taken to the laboratory where achenes were sorted, counted, and inspected for damage. Achenes were classified as dead if at least 25% of the proximal end of an achene was damaged. Herbivory was severe in 21 capitula, which resulted in the loss of most or all achenes within those capitula. Therefore, the number of disc, intermediate, and ray achenes produced and consumed in those capitula could not be counted and was estimated. We used a linear regression that related the number of disc, intermediate, and ray achenes to capitulum diameter. We obtained these data from 65 capitula on experimental plants that had experienced no larval herbivory. We used this model to estimate the total number of disc, intermediate and ray achenes produced in capitula that experienced extreme herbivory using their diameters. We also used these plants to estimate yield in our model described below. The proportion of consumed achenes for these 21 capitula was calculated using the estimated totals and the number of viable (unconsumed) achenes.

To account for the random effects of the date of capitulum opening, date of inoculation, and capitulum diameter in our analysis, the proportion of achenes

consumed within and among oviposition treatments was analyzed using a Bayesian linear mixed model with Markov chain Monte Carlo parameter estimation in the R version 3.0.2 (R Core Team, 2014) package {MCMCglmm} (Hadfield, 2010). This analysis is particularly useful when analyzing three or more random effects (Bolker *et al.*, 2009). All proportions were arcsine-transformed before analysis (Sokal and Rohlf, 1981). We ran each analysis for 3,000,000 iterations with a burn-in of 2,500,000 and a thinning interval of 100. This generated 5000 samples from each chain to calculate posterior mean ± SD [SD], posterior mode, and 95% credible intervals [lower CI–upper CI], and pMCMC probability values. Terms were considered statistically significant when pMCMC values calculated in MCMCglmm were less than 0.05 and 95% CIs did not span zero (Hadfield, 2010). We used an inverse gamma prior for random effects (V = 1, nu = 0.002, Hadfield, 2010). We ran each analysis three times using the Gelman-Rubin potential scale reduction statistic (PSR) to compare within and between chain variance (Gelman and Rubin, 1992). Convergence is met when PSR < 1.1, and in all our analyses PSR was always less than or equal to 1.01. The reported posterior means, confidence intervals, and pMCMC values were obtained by combining posterior distribution of (co)variance matrices [VCV] and posterior distribution of location effects [Sol] from each of the three models into one model.

Differences in larval survival after oviposition treatments were analyzed with a Kaplan–Meier log-rank test in R version 3.0.2 (R Core Team, 2014).

Seed bank mortality and seedling emergence—

To determine emergence proportions, seed bank mortality, and seed bank longevity, we sowed *G. ciliata* achenes in the field, monitored seedling emergence, and

retrieved ungerminated achenes from the soil at the end of the experiment. Because dormancy breaking requirements of intermediate and ray achenes were not known, we tested two durations of cold storage (stratification) and one physical manipulation (scarification) to determine their effects on germination. Whole, ripe primary capitula were collected 23 September 2010 from approximately 50 individuals located in an old field population north of Norman, OK (35.2482, –97.4781). Capitula were placed in coin envelopes and returned to the laboratory where achene morphs from all individuals were sorted and pooled. From each pool of achenes, 2400 achenes were randomly divided into four manila coin envelopes. One envelope of each achene type was randomly chosen as the control group and stored at room temperature until planting. A second envelope of each achene type was stored at 5°C for 30 d. A third envelope of each achene type was stored at ambient room temperature for 22 d, and then stored at 5°C for 15 d. Achenes in remaining envelopes were scarified at the proximal end using a razor blade and dissecting scope. Pericarps were not pierced during the process of scarification. Achenes were returned to envelopes immediately after scarification and stored at room temperature until planting.

All envelopes were removed from storage conditions 30 October 2010 and sown into buried pots filled with native soil in a rodent exclosure subplot (20 × 10 m) located at KAEFS. Control, scarified, and stratified achenes were randomly assigned to 15 plastic 3.78 L pots. Assignment of pots to treatments was conducted by assigning each pot (60 total) a randomly drawn number, without replacement, between one and 60. The numbered pots were ordered sequentially from lowest to highest along two parallel transect lines 2 m apart. Pots were buried approximately 15 cm apart from another.

Forty achenes of a single type and treatment were sown into each pot. All achenes were lightly covered with native soil, but not completely buried, to mimic primary seed dispersal and minimize secondary dispersal by wind. Because we could not determine the exact time of germination (i.e., when the radicle protruded through the fruit wall), we used seedling emergence as an indirect indicator of this event. Emergence was defined when cotyledons completely opened above the soil surface. We observed seedling emergence every 3–5 d for the first 2 mo after planting, every 6–9 d for the next 3 mo, and once per month thereafter for a total of 558 d. After emerging, seedlings were removed from pots. The last measure of seedling emergence was 3 May 2012. At that time, the upper 8 cm of soil from each pot was taken to the laboratory, sieved, and ungerminated achenes were tested for viability using tetrazolium staining (Porter *et al.*, 1947).

Statistical analysis of seed bank mortality and seedling emergence—

Seed-bank mortality was calculated by subtracting the number of germinated seeds and viable but ungerminated achenes found in the soil after 558 d from the total number of sown achenes. Mean percentage achene mortality per pot was arcsine-transformed to normalize variances (Sokal and Rohlf, 1981). Differences in percent achene mortality among achene types and treatments were tested through a one-way ANOVA using R version 3.0.2 Directional effects between achene types and treatments were examined using Tukey's HSD post hoc test.

Effects of stratification and scarification on seedling emergence were analyzed using Cox Proportional Hazard models (Cox and Oakes, 1984) with Heaviside functions in R version 3.0.2 (R Core Team, 2014). The hazard function calculates the

instantaneous probability of "failure" per unit of time (Kleinbaum and Klein, 2012) where failure is defined as a seed that emerges (i.e., "fails" to remain dormant in the seed bank). Treatment effects are reported through a hazard ratio (HR), which is the ratio of two hazard functions. For example, when the seedling emergence probability, $\hat{h}$, after an experimental treatment (A) is equal to $\hat{h}$ of the control (B), then HR = $\hat{h}(A)$ / $\hat{h}(B)$ = 1.0, indicating no difference in emergence rates. In contrast, when $\hat{h}(A)$ = 10[$\hat{h}(B)$], and HR = $\hat{h}(A)$ / $\hat{h}(B)$, then HR = 10, indicating that experimental treatment emergence is 10 times more likely than control emergence. Conversely, if $\hat{h}$ (B) = 10[$\hat{h}$ (A)] and HR = $\hat{h}(A)$ / $\hat{h}(B)$, then HR = 0.1, and experimental treatment emergence is one tenth as likely as control emergence.

Heaviside functions obtain separate hazard ratios for chosen time intervals (Kleinbaum and Klein, 2012). We divided our data set into two time periods, which separated Year 1 (Fall 2010 and Spring 2011) from Year 2 (Fall 2011 and Spring 2012) emergence. We combined spring and fall emergence events because those seedlings will flower together the following summer–fall. To account for multiple repeated measurements at the pot and seed level, we implemented cluster terms to calculate robust standard errors for coefficient estimates that account for nonindependence of observations (Kleinbaum and Klein, 2012). Germination probabilities and 95% confidence intervals were derived for observed emergence fractions by the Kaplan–Meier method using the function survfit in R.

Herbivory-independent optimal achene proportion model—

We first tested classical predictions of bet-hedging theory for heterocarpic *G. ciliata* by calculating individual fitness under simulated environments that differed in

the frequency of postgermination seedling mortality. We refer to intermediate and ray achenes collectively as dormant achenes based on the findings regarding seedling emergence described above. The findings regarding seed-bank longevity presented in this paper suggest that very few achenes remain viable in the seed bank beyond the spring of year 2, therefore our model uses a 2-yr seed bank. For each simulated environment, we incrementally changed the proportion of dormant (intermediate and ray) and nondormant (disc) achenes produced in a capitulum to determine optimal allocation strategies that maximized geometric mean fitness for each simulated environment. We used predispersal and postdispersal mortality results in a discrete deterministic growth model to calculate the net reproductive output (R_0) as it corresponds to the *G. ciliata* life cycle (Fig. 2):

$$R_0 = \left\{ \begin{array}{l} \left[Y_\alpha (1 - D_\alpha) \mathrm{G1}_\alpha E_t \right] + \left[\left[Y_\alpha (1 - D_\alpha)(1 - \mathrm{G1}_\alpha) \right] \mathrm{G2}_\alpha E_{t+1} \right] \\ + \left[\left[Y_\alpha (1 - D_\alpha)(1 - \mathrm{G1}_\alpha)(1 - \mathrm{G2}_\alpha) \right] E_{t+2} \right] \end{array} \right\} + \left[Y_\beta (1 - D_\beta) \mathrm{G1}_\beta E_t \right] \quad \text{(Eq. 1)}$$

where the net individual reproductive output (R_0) is calculated from the total yield of dormant (Y_α) and nondormant (Y_β) achenes, the fraction of dormant and nondormant achenes that become inviable in the seed bank are (D_α and D_β, respectively), the fraction of seedlings that emerge from dormant and nondormant achenes in the same season as dispersal ($G1_\alpha$ and $G1_\beta$, respectively), the fraction of seedlings that emerge from dormant achenes 1 year after dispersal ($G2_\alpha$), and 2 years after dispersal ($G3_\alpha$), and the fraction of seedlings that survive to reproduction after emergence from the seed bank (E). Emergence fractions $G1_\alpha$, $G1_\beta$, and $G2_\alpha$ represent the observed average emergence in the field, and $G3_\alpha$ is the assumed emergence of the viable proportion of achenes

recovered from the seed bank in the spring of the second year after dispersal. Following the methods of Cohen (1966), all seedlings that germinate in a particular year either survive in a favorable year ($E = 1$) or perish in an unfavorable year ($E = 0$). Individual reproductive output was modeled for 100 yr, and because seed bank longevity is limited to 2 years as described, the occurrence of consecutive unfavorable years after each favorable year is the worst theoretical environment (e.g., a repeating sequence of consecutive bad years followed by a good year 0, 0, 1). We compared 14 environments ranging in frequency of unfavorable years from 0 to 0.65 by increments of 0.05.

Yield was assumed to be equal for all surviving seedlings, irrespective of achene type, and each surviving individual produced a fixed number of capitula (30) each having 40 ray florets. The total achene number per capitulum, was estimated from the linear relationship between ray floret number and total achenes ($y = 7.4x + 36.8$, Multiple $R^2 = 0.48$). This total was partitioned into a proportion of dormant (Y_α) and nondormant (Y_β) achenes. The proportion of nondormant to dormant achenes was changed in increments of 0.1, starting at 0, creating 101 unique proportions. Each unique proportion remained constant for the 100-yr duration of each simulated environment, and all 101 proportions were tested for each environment. The proportion of dormant to nondormant achenes that maximized geometric mean fitness in a given environment was chosen as the optimal strategy.

Herbivore-dependent optimal achene proportion model—

We next examined the influence of predispersal achene predation on theoretical expectations of the proportions of dormant to nondormant achenes relative to

environmental quality by modifying Eq. (1) so that yield of dormant achenes after herbivory (YH_{α}) is

$$YH_{\alpha} = Y_{\alpha} - (Y_{\alpha}H_{\alpha}), \quad (2)$$

and the yield of nondormant achenes after herbivory (YH_{β}) is

$$YH_{\beta} = Y_{\beta} - (Y_{\beta}H_{\beta}), \quad (3)$$

where H_{α} and H_{β} represent, respectively, the proportion of dormant and nondormant achenes consumed. In natural populations, the total proportion of consumed achenes will vary among individuals and years due to fluctuations in moth abundance, oviposition timing, and capitulum production. Therefore, we created scenarios that account for some of this natural variation. We used a moderate *S. mortua* influence scenario to mimic when females do not oviposit at times that maximize larval survival (Thompson, 1988c; Thompson and Pellmyr, 1991). In this herbivory scenario, H_{α} and H_{β} were represented by the average of the dormant and nondormant achenes in the D_4, D_7, and D_{14} treatments that were consumed from capitula in which larvae consumed at least one achene (n = 24). The scenario that occurs when a proportion of capitula do not experience any herbivory is not explicitly run, but can be inferred by the difference between results calculated from the herbivore-independent and moderate-herbivory models. To mimic the scenario when all capitula experience high herbivory because few capitula are produced or moth abundance is high and females make optimal oviposition choices, we created an extreme-herbivory scenario. The values of H_{α} and H_{β} in the extreme-herbivory scenario were represented by the highest average proportion of consumed dormant and nondormant achenes in our manipulative study (D_4-inoculated

capitula that lost at least one achene, $n = 11$). To evaluate the temporal variability of extreme herbivory, we incrementally varied the frequency of extreme herbivory, from 0.01 to 1 by 0.01 increments, in each of the previously described environments and achene proportions.

Chapter 3: Results

Predispersal achene survival—

Initial comparisons among achene types revealed a significantly higher overall mean percentage of consumption of disc achenes (21.9% ± 3.9, Fig. 3) than of the intermediate (8.9% ± 2.7, CI: 0.12–0.32, pMCMC < 0.00007) or ray achenes (6.1% ± 2.4, 95% CI: 0.19–0.4003, pMCMC < 0.00007). Disc achenes were also consumed at significantly higher levels than intermediate and ray in the D_4 and D_7 oviposition treatments. In the D_4 treatment, the mean percentage of consumed disc achenes (58.6% ± 10.6) was significantly higher than the mean percentage of consumed intermediate (32.1% ± 9.7, CI: 0.018–0.71, pMCMC = 0.044), and ray achenes (20.4% ± 9.2, CI: 0.232–0.956, pMCMC = 0.003, Fig. 3). In the D_7 treatment, the mean percentage of consumed disc achenes (22% ± 6.8) was also significantly higher than for intermediate (1.2% ± 0.94, CI: 0.14–0.749, pMCMC = 0.009) and ray achenes (0.31% ± 0.31, CI: 0.16–0.81, pMCMC = 0.004, Fig. 3). There were no significant differences in consumption among achene types in the D_1, D_{14}, and D_{21} treatments (Fig. 3).

Total achene consumption among oviposition treatments was significantly higher in the D_4 treatment (42.3% ± 9.2) than all other oviposition treatments (Table 1).

Total achene consumption was not significantly different among D_1 (13.7% ± 7.5), D_7 (9.9% ± 3.0), D_{14} (6.4% ± 2.7), or D_{21} (0.2% ± 0.13) treatments (Table 1). Disc achene consumption among treatment groups was consistent with this trend. The mean percentage of disc achenes consumed was significantly higher in the D_4 treatment (58.6% ± 10.6) than all other treatments (Table 1). The mean percentage of intermediate achenes consumed was significantly higher in the D_4 treatment only (32.1% ± 9.7) than the D_7, D_{14}, and D_{21} treatments (Table 1). No significant differences were found among oviposition treatments in the mean percentage of consumed ray achenes (Table 1).

Larval survival—

The Kaplan–Meier log-rank test detected a significant difference ($\chi^2 = 12.8$, df = 4, $p = 0.012$) in *S. mortua* larval survivorship across oviposition treatments. Mean larval survival was significantly higher in the D_4 treatment (mean ± SE: 0.733 ± 0.114, $n = 15$, df = 1, $\chi^2 = 12.1$, $p = 0.0005$) than in the D_{21} (0.177 ± 0.093, $n = 17$) and D_1 treatments (0.333 ± 0.122, $n = 15$, df = 1, $\chi^2 = 4.66$, $p = 0.031$). Mean larval survival in the D_7 (0.438 ± 0.124, $n = 16$) treatment was also significantly higher than D_{21} treatment (0.177 ± 0.093, $n = 17$, df = 1, $\chi^2 = 4.12$, $p = 0.042$). Other pairwise comparisons, including the D_{14} (0.400 ± 0.126, $n = 15$) treatment, did not differ significantly.

Seed-bank mortality and seedling emergence—

One-way ANOVA detected significant differences in total seed bank mortality among disc, intermediate, and ray achenes seeds ($F_{2, 177} = 196.731$, $p < 0.0001$). Tukey's HSD revealed that disc achene seed bank mortality (mean ± SE: 29.3% ± 2.1) was significantly lower than intermediate (72.5% ± 1.6, $p < 0.001$) and ray (80.1% ±

1.5, $p < 0.001$) achene mortality in the seed bank. Furthermore, intermediate achene seed bank mortality was also significantly lower than ray achene seed mortality ($p < 0.001$). No significant differences in seed mortality were detected among scarification and stratification treatments or for achene type × seed storage treatment interactions.

No viable disc achenes remained in the seed bank at the end of the experiment. In contrast, a small fraction of viable ray (1.67% ± 0.28) and intermediate (2.3% ± 0.32) seeds remained in the seed bank after the spring of year 2 (558 d after sowing). No statistical differences in viability were found among stratification and scarification treatments of intermediate and ray achenes.

The odds of intermediate and ray achene seedling emergence were significantly lower than disc achene seedling emergence (intermediate HR = 0.01, $P < 0.0001$, ray HR = 0.008, $P < 0.001$, Fig. 4A) in the fall and summer of year 1 (1–260 d after sowing [DAS]). No further disc achene seedlings emerged after this period. Ray and intermediate seedling emergence did not differ significantly in year 1 (1–260 DAS HR = 0.778, $P = 0.067$) or year 2 (261 – 558 DAS HR = 0.955, $P = 0.480$). The largest ray and intermediate seedling emergence events occurred in the fall of year 2, but smaller emergence events were also observed in the fall of year 1, and spring of year 1 and 2 (Fig. 4A-E). The odds of intermediate and ray seedling emergence were significantly lower than disc achene seedling emergence in all treatments (Fig. 4B-E). However, ray achene seedlings had significantly lower odds of emergence than did intermediate seedling emergence before the summer of year 1 in the control (HR = 0.345, $P = 0.0045$, Fig. 4B) and 15-d stratified treatment (HR = 0.559, $P = 0.018$, Fig. 4C).

In comparison to control seedling emergence, the 30-d cold stratification significantly reduced the odds of seedling emergence in year 2 (HR = 0.78, $P = 0.007$) but not in year 1. However, mean disc seedling emergence was unaffected by stratification (15-d HR = 0.95, $P = 0.39$, 30-d HR = 0.94, $P = 0.38$) or scarification (HR = 0.93, $P = 0.28$; Appendix B, see Supplemental Data with the online version of this article). Intermediate seedling emergence were mostly unchanged; however, the 30-d stratification significantly reduced the odds of emergence in year 1 (HR = 0.57, $P = 0.04$) and year 2 (HR = 0.7, $P = 0.005$; see Appendix B). For ray achenes, the odds of emergence were higher only in year 1, after the 15-d stratification (HR = 2.11, $P = 0.04$), 30-d stratification (HR = 2.47, $P = 0.01$), and scarification (HR = 3.75, $P = 0.0002$; see Appendix B).

Optimal achene proportion models—

Optimal proportions of nondormant (disc) achenes calculated by the herbivory-independent model were negatively and linearly related to the probability of unfavorable years in an environment (Fig. 5). Herbivory reduced nondormant achene proportions of optimal achene allocation strategies in comparison to herbivore-independent strategies (Fig. 5). However no difference was observed in environments that lacked unfavorable years and experienced extreme herbivory frequencies of 0.87 or less, for which optimal strategies consisted of 100% nondormant achenes, as in the herbivore-independent model (Fig. 5). The largest difference in achene allocation proportion was 1.0 (i.e., 100%), in environments that lacked unfavorable years and experienced extreme herbivory frequencies ranging from 0.94 to 1.0 because a 100% dormant achene strategy maximized mean geometric fitness (Fig. 5). However,

homocarpy (i.e., 0% or 100% nondormant achenes) was not always an optimal strategy in environments that lacked unfavorable years; rather, various degrees of heterocarpy were optimal when the frequency of extreme herbivory ranged from 0.88 to 0.93 (Fig. 5). Decreases in nondormant achenes in optimal allocation strategies due to predispersal herbivory were larger and more variable in environments with low frequencies of unfavorable years (i.e., ranging from 0.00 to approximately 0.35), in comparison to environments with unfavorable year frequencies of 0.40 or higher. In particular, in environments with unfavorable year frequency 0.35 or lower nondormant achene allocation proportions decreased by 0.0–0.11, 0.0–0.12, 0.0–0.15, 0.00–0.23, 0.0–038, and 0.23–1.0 under extreme herbivory frequencies of 0.0, 0.20, 0.40, 0.60, 0.80, and 1.0 respectively (Fig. 5). In contrast, in environments with unfavorable year frequencies of 0.40 or higher nondormant achene allocation proportions decreased by 0.03–0.07, 0.04–0.09, 0.04–0.11, 0.06–0.12, 0.07–0.12, and 0.09–0.17 under moderate (i.e., 0.0 extreme herbivory), 0.20, 0.40, 0.60, 0.80, and 1.0 extreme herbivory, respectively (Fig. 5).

Chapter 3: Discussion

Heterocarpy is an adaptive bet-hedging strategy that offsets postdispersal seed and seedling mortality risks by producing offspring that differ in dormancy and dispersal ability, resulting in increased geometric mean fitness (Venable, 1985b; Imbert, 2002). Our study provides evidence of an additional important aspect of bet-hedging in a heterocarpic system, offsetting predispersal seed herbivory. Nondormant (disc) *G. ciliata* achenes offset seed bank mortality risks through immediate germination after dispersal, but are highly susceptible to predispersal herbivory. They experience minimal

mortality in the seed bank due to their rapid germination, but are immediately exposed to potential postgermination mortality due to highly variable water availability in the fall (Illston *et al.*, 2004) and low temperatures during the winter. In contrast, dormant (intermediate and ray) *G. ciliata* achenes minimize predispersal herbivory and delay exposure to potential postgermination mortality risks by approximately one growing season. However, because of this germination delay, they are more susceptible to mortality in the seed bank.

In regard to specific selection agents, the vulnerability of *G. ciliata* achenes to predispersal mortality was strongly dependent on the timing of *S. mortua* ovipositing in capitula. In comparison with capitula on day 4 after opening (D_4), achene consumption was significantly lower in the D_1, D_7, D_{14}, and D_{21} treatments, suggesting that food availability for neonates and later instars is affected by the synchronization of oviposition, larval maturation, and capitulum development. For all instars, the availability and efficiency of food handling is affected by mandible development (Chapman, 1995). Neonates, however, mainly feed on pollen (Zalucki *et al.*, 2002), which, in *G. ciliata* and many other composites, is released by florets that open centripetally on the capitulum. The *S. mortua* neonates placed into D_1 capitula could likely not access their primary nutrition source for several days without neonate migration within a capitulum, thereby possibly causing high neonate mortality and low achene consumption in the D_1 treatment. Neonates in the D_4 and D_7 treatments would have been exposed to fresh pollen at the time of oviposition, leading to increased neonate survival and total achene consumption. Neonates from the D_{14} and D_{21} would have limited access to fresh pollen or floral parts, possibly causing the increased

likelihood of larval mortality and low consumption of achenes. The optimal *S. mortua* time for neonate hatching would be at between D_4 and D_7, and possibly 1 or 2 days before D_4 or after D_7, when pollen is accessible to neonates.

The synchronization between flowering and oviposition is central to the success of *S. mortua* neonates but can also affect the survival of older instars. Older instars, although capable of consuming pollen, typically consume developing ovaries, pericarps, and seeds (Louda, 1982a, 1982b,1983; Maron *et al.*, 2002). Seeds and pericarps become unavailable if pericarps harden (lignify) before larval mandibles are sufficiently developed. Achene pericarp cells begin to harden 5 days postanthesis in *Helianthus annuus* L. (Lindström *et al.*, 2007), suggesting that in *G. ciliata,* pericarp hardening had begun by D_7 in ray and outer intermediate achenes, and by D_{14} in disc and centrally located intermediate achenes. In D_1 capitula, disc, intermediate, and ray achenes are equally vulnerable to later instars, and larval mandible development is likely not lagging behind pericarp development. Still, achene consumption was low, possibly due to copious resin production, a prominent characteristic of developing *Grindelia* capitula (Hoffmann *et al.*, 1984; Timmermann and Hoffmann, 1985; McLaughlin and Linker, 1987), which could provide additional protection to developing florets. Larval mandible development in D_4 capitula was likely synchronized with achene pericarp development, giving later instars access to mature seed before pericarp lignification, resulting in severe disc, intermediate, and ray achene consumption. By D_7, ray and intermediate achene pericarp hardening may have been sufficiently ahead of larval mandible development, resulting in drastically lower consumption of intermediate and ray achenes. However, disc achenes likely remained vulnerable because their pericarp

development is delayed compared with exterior ray and intermediate achenes, and the disc achene pericarp is softer and composed of fewer layers of lignified cells. Larval and achene development was further offset in D_{14} capitula, resulting in the loss of only a few disc achenes, likely because the pericarps achenes were sufficiently hardened. However, by D_{21}, even disc achenes experienced nearly no consumption, likely due to protection provided by mature pericarps and a reduced time for achene consumption due to achene dispersal approximately 2 weeks later.

Emergence of *G. ciliata* seedlings from all achene types occurred predominantly between September and November of year 1 (2010) and year 2 (2011), suggesting that fall is the optimal time for *G. ciliata* seed germination and seedling establishment. Disc achenes displayed immediate opportunistic recruitment, with very few disc achenes remaining in the seed bank for longer than 12 d after sowing. Ray and intermediate seedlings offset the risks of immediate emergence by spreading emergence over the next year. Optimal seedling emergence times are shaped by mortality-inducing factors (Donohue *et al.*, 2010), which, for seedlings, are commonly associated with severe drought at shallow soil depth (Kitajima and Fenner, 2000). Survival probability of *G. ciliata* seedlings is likely affected by their ability to cope with receding soil moisture levels, which, across Oklahoma, become most severe between August and November (observed *G. ciliata* emergence times), following an enhanced soil drying phase that typically lasts from mid June until late August (Illston *et al.*, 2004). Soil moisture levels at shallow depths (i.e., 5 cm and 25 cm) between July and November of 1997–2002 were highly variable (Illston *et al.*, 2004), suggesting that all *G. ciliata* seedlings are at risk from unpredictable fall soil moisture levels. Intermediate and ray achenes can offset

this risk by staggering seedling emergence over several events lasting from early to late fall (Fig. 4; Appendix B). The mortality risks associated with immediate germination can also be offset by multiple flowering and achene dispersal events (Ritland, 1983), resulting in several disc seedling emergence events in the year of dispersal.

Oklahoma soil moisture remains elevated throughout winter and early spring. However, few *G. ciliata* seedlings emerged in the winter and spring, suggesting that seeds are not cued to emerge during this time. Physiological or physical mechanisms, or a combination of both, may be inhibiting spring seedling emergence. The thick pericarp of intermediate and ray achenes functions to inhibit water uptake (J. P. Gibson unpublished data) and could physically restrict radicle protrusion, as was shown in ray achenes of *Grindelia squarrosa* (McDonough, 1975). Although we carefully screened achenes before sowing, it is also possible that some viable seeds were contained in pericarps that sustained *S. mortua* damage during development, which may have jeopardized the integrity of the pericarp and led to early germination in the fall or spring of year 1 (Koptur, 1998). On the other hand, the physiological condition of seeds might also have affected germination. It is possible that intermediate and ray achene germination was inhibited by a conditional dormancy, which prevents germination unless very specific germination inducing conditions (e.g., a threshold minimum or maximum soil temperature or moisture) were experienced (Baskin and Baskin, 1998). Variation in conditional dormancy among achenes is not unexpected due to genetic and epigenetic differences among individuals.

It is not clear why spring conditions may not be favorable for *Grindelia ciliata* seedling emergence. Seedlings emerging in the spring may have insufficient time, in

comparison to fall emerged seedlings, to store energy for root growth or to establish deeper root systems before soil moisture levels become unsuitable for plant growth in the summer (fractional water index < 0.3). Soil moisture recession typically occurs in Oklahoma by July at shallow depths (5 – 25 cm) and by August at deeper depths (60 – 75 cm; Illston *et al.*, 2004). Soil water levels then rise to levels tolerable to seedlings after November.

Traditional bet-hedging model predictions have had mixed support from experimental studies, and recent studies have revealed a great need to focus on traits not considered in traditional models (Wilbur and Rudolf, 2006; Ellner and Rees, 2007; Morris *et al.*, 2008; Rees and Ellner, 2009; Shefferson, 2009; Rose *et al.*, 2009, Metz *et al.*, 2010, Childs *et al.*, 2010). For example, Gremer and Venable (2014) showed that incorporating density dependence into a traditional bet-hedging model improved the fit between observed and predicted germination fractions. In that example, the traditional (density-independent) model overestimated predictions of optimal germination fractions, which is equivalent to overestimating the proportion of nondormant achenes in a capitulum in our study. Our traditional herbivore-independent bet-hedging model also overestimated the proportion of nondormant achenes in optimal strategies in almost all simulated predispersal mortality scenarios (Fig. 5). Furthermore, our findings support the hypothesis that predispersal mortality could lead to the evolution and maintenance of the bet-hedging strategy heterocarpy. Our study also highlights the idea that selective pressures have additive effects. For example, in our study predispersal and postgermination mortality both select for dormant achene production and the additive

effects strongly influenced expected optimal strategies, in particular when both selective pressures are moderate or weak (Fig. 5).

Conclusion

Our study is the first to show theoretical and empirical evidence that predispersal mortality is a significant factor shaping the adaptive value of heterocarpy in *G. ciliata*. However, due to the ubiquitous nature of seed mortality due to insect herbivory, it is likely a significant evolutionary pressure affecting reproductive bet-hedging not only heteromorphic species but all plants. Additionally, predispersal mortality has not been considered in previous theoretical and empirical investigations of heterocarpy or other bet-hedging strategies. Therefore, further evaluations of this mortality risk in shaping plant reproductive ecology in other species are needed to fully understand the evolutionary biology and adaptive value of other reproductive bet-hedging strategies in other environments.

Statement of conflict of interest

The authors are not aware of any conflict of interest.

Data Repository

Population growth models and data sets are deposited at http://dx.doi.org/10.5061/dryad.7c63h.

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Chapter 3: References

Bachmann K., K. L. Chambers, and H. J. Price. 1984. Genetic components of heterocarpy in *Microseris* hybrid B87 (Asteraceae, Lactuceae). *Plant Systematics and Evolution* 148: 149–164.

Baker, G. A. and D. J. O'Dowd. 1982. Effect of parent plant density on the production of achene types in the annual *Hypochoeris glabra*. *Journal of Ecology* 70: 201–215.

Baskin J. M. and C. C. Baskin. 1979. Studies on the autecology and population biology of the monocarpic perennial *Grindelia lanceolata*. *American Midland Naturalist* 102: 290-299.

Baskin C. C. and J. M. Baskin. 1998. *Seeds: ecology, biogeography, and evolution of dormancy and germination*. San Diego, CA, USA: Academic Press.

Bolker B. M. *et al.* Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution Volume 24, Issue 3, 127-135.

Brown J. S. and D. L. Venable. 1986. Evolutionary ecology of seed bank annuals in temporally varying environments. *American Naturalist* 127: 31-47.

Byers B. A. 1989. Biology and immature stages of *Schinia masoni* (Noctuidae). *Journal of Lepidopterists' Society* 43(3): 210-216

Chapman R.F. 1995. Mechanics of Food Handling by Chewing Insects, *In* Chapman R.F., de Boer G., *Mechanisms in Insect Feeding*, Springer US. p. 3-31.

Childs D. Z., C. J. E. Metcalf, and M. Rees. 2010. Evolutionary bet hedging in the real world: empirical evidence and challenges revealed by plants. *Proceedings of the Royal Society Biological Sciences* 277: 3055–3064.

Cohen D. 1966. Optimizing reproduction in a randomly varying environment. *Journal of Theoretical Biology* 12: 119 – 129.

Cooper W. S. and R. H. Kaplan. 1982. Adaptive coinflipping— a decision-theoretic examination of natural-selection for random individual variation. *Journal of Theoretical Biology* 94: 135–151.

Cox, D. R. & D. Oakes. 1984. Analysis of Survival Data. London: Chapman and Hall

Crean A. J. and D. J. Marshall. 2009. Coping with environmental uncertainty: dynamic bet hedging as a maternal effect. *Philosophical Transactions of the Royal Society of London*. Series B, Biological Sciences 364: 1087–1096.

de Clavijo E. R. 1994. Heterocarpy and seed polymorphism in *Ceratocapnos heterocarpa* (Fumariaceae*). International Journal of Plant Sciences* 155: 196-202.

Donohue K., R. R. de Casas, L. Burghardt, K. Kovach, and C. G. Willis. 2010. Germination, post germination adaptation, and species ecological ranges. *Annual Review of Ecology, Evolution, and Systematics* 41: 293–319.

Ellner S. P. and A. Shmida. 1984. Seed dispersal in relation to habitat in the genus *Picris* (Compositae) in Mediterranean and arid regions. *Israel Journal of Botany* 33: 25–39.

Ellner S. 1986. Germination dimorphisms and parent-offspring conflict in seed germination. *Journal of Theoretical Biology* 123: 173-185.

Ellner, S. P. and Rees, M. 2007. Stochastic stable population growth in integral projection models: theory and application. *Journal of Mathematical Biology* 54: 227 – 256.

Evans M. E. K. and J. J. Dennehy. 2005. Germ banking: bet hedging and variable release from egg and seed dormancy. *The Quarterly Review of Biology* 80: 431–451.

Fenner M., L.E. Cresswell, R.A. Hurley, and T. Baldwin. 2002. Relationship between capitulum size and pre-dispersal predation by insect larvae in common Asteraceae. *Oecologia 130: 72-77.*

Gillespie J. H. 1974 Natural selection for within generation variance in offspring number. *Genetics* 76: 601 – 606.

Gibson J. P. 2001. Ecological and genetic comparison between ray and disc achene pools of the heteromorphic species *Prionopsis ciliata* (Asteraceae). *International Journal of Plant Sciences* 162: 137–145.

Graham J. K., M. L. Smith, and A. M. Simons. 2014. Experimental evolution of bet hedging under manipulated environmental uncertainty in *Neurospora crassa. Proceedings of the Royal Society Biological Sciences* 22: 281

Gremer J. R. and D. L. Venable. 2014. Bet hedging in desert winter annual plants: optimal germination strategies in a variable environment. *Ecology Letters* 17 (3): 380-387

Grote, A. R. 1874. *List of the Noctuidae of North America*.

Goeden R. D. and D. W. Ricker. 1977. Establishment of *Rhinocyllus conicus* on milk thistle in southern California. *Weed Science* 25: 288-292.

Goeden R. D. and D. W. Ricker. 1978. Establishment of *Rhinocyllus conicus* on Italian thistle. *Environmental Entomology* 7: 787-789.

Gutterman Y. 1994a. Long-term seed position influences on seed germinability of the desert annual, *Mesembryanthemum nodiflorum L. Israel Journal of Plant Sciences* 42: 197 – 205.

Hadfield J. D. 2010. MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. *Journal of Statistical Software* 33(2): 1-22. URL http://www.jstatsoft.org/v33/i02/.

Hardwick D. F. 1971. The life history of *Schina separata* (Noctuidae). *Journal of Lepidopterists' Society* 25: 177-180

Harper J. L. 1969. The role of predation in vegetation diversity. Brookhaven Symposiumin Biology, 22: 48-62.

Harper J. L. 1977. *Population biology of plants*. London: Academic Press

Hoffmann J. J., B. E. Kingsolver, S. P. McLaughlin, and B. N. Timmermann. 1984. Production of resins by arid-adapted Astereae. *In* B. N. Timmermann, C. Steelink, and F. A. Loewus. *Phytochemical Adaptations to Stress*, p. 251–271. Plenum, New York.

Huffaker C. B. and C. E. Kennet. 1959. A ten-year study of vegetational changes associated with biological control of Klamath Weed. *Journal of Range Management* 12: 69-82.

Illston B., J. Basara, and K. Crawford. 2004. Seasonal to Interannual Variations of Soil Moisture Measured in Oklahoma. *International Journal of Climatology* 24: 1883-1896

Imbert E. 2002. Ecological consequences and ontogeny of seed heteromorphism. *Perspectives in Plant Ecology, Evolution, and Systematics* 5: 13–36.

Jaimand K. and M. B. Rezaee. 1996. Variability in seed composition due to plant population and capitula zones of sunflower. *Agrochimica* 40: 48–54.

Janzen D. H. 1971. Seed predation by animals. *Annual Review of Ecology and Systematics* 2: 465-492.

Kitajima K. and M. Fenner. 2000. Ecology of seedling regeneration. In: Fenner M. *Seeds: the ecology of regeneration in plant communities*. Wallingford, UK: CABI Publishing, 331–359.

Kleinbaum D. G. and M. Klein. 2012. *Survival Analysis*. Springer Science Inc., New York.

Kolb A., J. Ehrlén, and O. Eriksson. 2007. Ecological and evolutionary consequences of spatial and temporal variation in pre-dispersal seed predation. *Perspectives in Plant Ecology, Evolution, and Systematics* 9: 79-100.

Koptur, S. 1998. Is a damaged seed a dead seed? Consequences of seed damage in the common vetch (Vicia sativa L.) and its major herbivore (Cydia spp.). *American Midland Naturalist* 140 (2): 393-396.

Lafontaine J.D., Schmidt B.C., 2010. Annotated check list of the Noctuidae (Insecta, Lepidoptera) of North America north of Mexico. *ZooKeys* 40: 1-239

Lindström L. I., C. N. Pellegrini, and L. F. Hernández. 2007. Histological development of the sunflower fruit pericarp as affected by pre- and early post-anthesis canopy shading. *Field Crops Research* 103: 229–238

Louda S. M. 1978. A test of predispersal seed predation in the population dynamics of *Haplopappus* (Asteraceae). Ph.D. thesis, The University of California, Riverside, California and San Diego State University San Diego, California.

Louda S. M. 1982. Distribution ecology: variation in plant recruitment over a gradient in relation to insect seed predation. *Ecological Monographs* 52: 25–41.

Louda S. M. 1982b. Limitation of the recruitment of the shrub *Haplopappus squarrosus* (Asteraceae) by flower- and seed-feeding insects. *Journal of Ecology* 70: 43-53

Louda S.M. 1983. Seed predation and seedling mortality in the recruitment of a shrub, *Haplopappus venetus* (Asteraceae), along a climatic gradient. *Ecology* 64(3): 511-521.

Mandák B. and P. Pyšek. 2001a. Fruit dispersal and seed banks in *Atriplex sagittata*: the role of heterocarpy. *Journal of Ecology* 89: 159–165.

Maron J. L., J. K. Combs, and S. M. Louda. 2002. Convergent demographic effects of insect attack on related thistles in costal vs. continental dunes. *Ecology* 83(12): 3382-3392.

Maxwell C. D., A. Zobel, and D. Woodfine. 1994. Somatic polymorphism in the achenes of *Tragopogon dubius*. *Canadian Journal of Botany* 72: 1282–1288.

McDonough N. T. 1975. Germination Polymorphism in *Grindelia squarrosa* (Pursh) Dunal. *Northwest Science* 49: 190–200.

McLaughlin S. P. and J. D. Linker. 1987. "Agronomic studies on gumweed: seed germination, planting density, planting dates, and biomass and resin production." *Field Crops Research* 15.3: 357-367.

Metz, J., Liancourt, P., Kigel, J., Harel, D., Sternberg, M. & Tielbörger, K. 2010. Plant survival in relation to seed size along environmental gradients: a long-term study from semi-arid and Mediterranean annual plant communities. *Journal of Ecology* 98: 697 – 704.

Morris, W. F. *et al.* 2008. Longevity can buffer plant and animal populations against changing climatic variability. Ecology 89: 19–25.

Murphy G. I. 1968. Pattern in life history and the environment. *The American Naturalist* 102 No. 927: 391-403.

Philippi T. 1993. Bet-hedging germination of desert annuals—beyond the 1st year. *American Naturalist* 142: 474–487.

Philippi, T. 1993b. Bet-hedging germination of desert annuals: variation among populations and maternal effects in *Lepidium lasiocarpum*. *American Naturalist* 142: 488-507.

Philippi T, J. Seger. 1989. Hedging ones evolutionary bets, revisited. *Trends Ecology and Evolution* 4: 41 – 44.

Pomplitz R. 1956. Die Heteromorphie der Früchte von *Calendula arvensis* unter besonderer Berücksichtigung der Stellungs und Zahlenverhältnisse. *Beiträge zur Biologie der Pflanzen* 32: 331–369.

Porter R. H., M. Durrell, and H. J. Romm. 1947. The Use of 2,3,5-Triphenyl-Tetrazoliumchloride as a Measure of Seed Germinability. *Plant Physiology* 22 (2): 149–159

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rees, M. & Ellner, S. P. 2009. Integral projection models for populations in temporally varying environments. Ecological Monographs 79: 575–594.

Ritland K. 1983. The joint evolution of seed dormancy and flowering time in annual plants living in variable environments. *Theoretical Population Biology* 24 (3): 213-243

Roff D. 2002. *Life history evolution*. Sinauer Associates, Sunderland, MA.

Rose, K. E., Atkinson, R. L., Turnbull, L. A. & Rees, M. 2009. The cost and benefits of fast living. *Ecology Letters* 12: 1379–1384

Salisbury E. J. 1942. *The Reproductive Ceapacity of Plants*. Bell, London.

Seger J. and H. J. Brockmann. 1987. What is bet-hedging? *Oxford Surveys in Evolutionary Biology* 4: 182 – 211.

Shefferson, R. P. 2009. The evolutionary ecology of vegetative dormancy in mature herbaceous perennial plants. *Journal of Ecology* 97: 1000–1009.

Simons A. M. 2011. Modes of response to environmental change and the elusive empirical evidence for bet hedging. *Proceedings of the Royal Society Biological Sciences* 278: 1601–1609.

Slatkin M. 1974. Hedging ones evolutionary bets. *Nature* 250: 704 – 705.

Sokal, R. R., and F. J. Rohlf. 1981.The Principles and Practice of Statistics in Biological Research. *Biometry*

Starrfelt, J. and Kokko, H. 2012. Bet-hedging—a triple trade-off between means, variances and correlations. *Biological Reviews* 87: 742–755.

Steyermark J. A. 1934. Studies in *Grindelia*, 2 a monograph of the North American species of the genus *Grindelia*. *Annals of the Missouri Botanical Garden* 21: 433-608

Thompson, J. N. 1988c. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomologia Experimentalis et Applicata* 47: 3-14

Thompson, J. N., and O. Pellmyr. 1991. Evolution of oviposition behavior and host preference in Lepidoptera. *Annual Review of Entomology* 36: 65-89

Timmermann B. N. and J. J. Hoffmann. 1985. Resins from *Grindelia*: a model for renewable resources in arid environments. *Plants Arid Lands*: 357-368.

Venable D. L. 1985a. The evolutionary ecology of seed heteromorphism. *American Naturalist* 126: 577–595.

Venable D. L. 1985b. Ecology of achene dimorphism in *Heterotheca latifolia* III. Consequences of varied water availibility. *Journal of Ecology* 73: 757–763.

Venable D. L. and D. A. Levin. 1985. Ecology of achene dimorphism in *Heterotheca latifolia*. 1. Achene structure, germination and dispersal. *Journal of Ecology* 73: 133–145.

Venable D.L., Búrquez A., Corral G., Morales E., Esponosa F. 1987. The Ecology of seed heteromorphism in *Heterosperma Pinnatum* in central Mexico. Ecology 68: 65-76.

Venable, D. L., Dyreson, E., & Morales, E. 1995. Population dynamic consequences and evolution of seed traits of *Heterosperma pinnatum* (Asteraceae). *American Journal of Botany* 82: 410-420.

Wilbur, H. M. & Rudolf, V. H. W. 2006. Life-history evolution in uncertain environments: bet hedging in time. *American Naturalist* 168: 398–411.

Zalucki MP, A. R. Clarke, and S. B. Malcolm. 2002. Ecology and behavior of first instar larval Lepidoptera. *Annual Review of Entomology* 47: 361–393.Zohary M. 1950. Evolutionary trends in the fruiting head of Compositae. *Evolution* 4: 103–109.

Zwick F. B. and J.R. Estes. 1981. Adaptation of the Life History of *Schinia mitis* (Lepidoptera: Noctuidae) to Its Host-Plant, *Pyrrhopappus*. *Journal of the Kansas Entomological Society*

Chapter 3: Tables

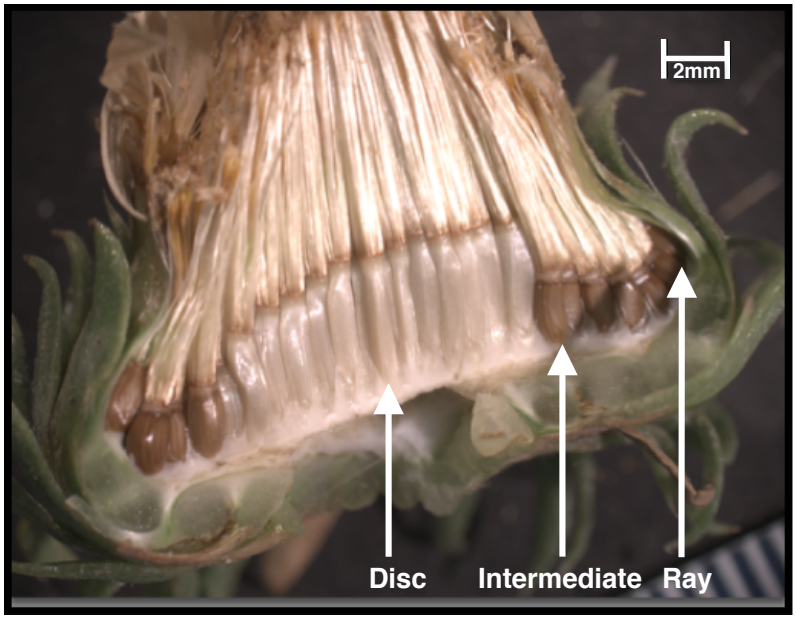
Table 1. Consumption differences among *Schinia mortua* larvae in the inoculation treatments for all *Grindelia ciliata* achenes combined or separately for disc, intermediate, and ray achenes.

Inoculation treatments with mean percent seeds consumed (±SE)		Estimate	Lower CI	Upper CI	pMCMC
All Achenes Combined					
D_1 (13.7% ± 7.5)	D_4 (42.3% ± 9.2)	−0.63	−1.2	−0.03	0.03
D_1	D_7 (9.9% ± 3.0)	−0.08	−0.6	0.5	0.77
D_1	D_{14} (6.4% ± 2.7)	0.01	−0.6	0.6	0.98
D_1	D_{21} (0.2% ± 0.13)	0.16	−0.4	0.7	0.58
D_4 (42.3% ± 9.2)	D_7 (9.9% ± 3.0)	0.55	0.04	1.2	0.05
D_4	D_{14} (6.4% ± 2.7)	0.64	0.13	1.01	0.03
D_4	D_{21} (0.2% ± 0.13)	0.78	0.26	1.3	0.005
D_7 (9.9% ± 3.0)	D_{14} (6.4% ± 2.7)	0.08	−0.42	0.56	0.74
D_7	D_{21} (0.2% ± 0.13)	0.22	−0.28	0.72	0.37
D_{14} (6.4% ± 2.7)	D_{21} (0.2% ± 0.13)	0.16	−0.33	0.61	0.57
Disc Achenes					
D_1 (17.2% ± 8.7)	D_4 (58.6% ± 10.6)	−0.77	−1.4	−0.13	0.02
D_1	D_7 (22% ± 6.8)	−0.21	−0.84	0.34	0.47
D_1	D_{14} (14% ± 6.7)	−0.01	−0.65	0.63	0.96
D_1	D_{21} (0.4% ± 0.3)	0.18	−0.43	0.78	0.55
D_4 (58.6% ± 10.6)	D_7 (22% ± 6.8)	0.52	0.03	1.15	0.05
D_4	D_{14} (14% ± 6.7)	0.75	0.13	1.41	0.02
D_4	D_{21} (0.4% ± 0.3)	0.95	0.39	1.5	0.001
D_7 (22% ± 6.8)	D_{14} (14% ± 6.7)	0.19	−0.36	0.79	0.50
D_7	D_{21} (0.4% ± 0.3)	0.39	−0.15	0.94	0.17
D_{14} (14% ± 6.7)	D_{21} (0.4% ± 0.3)	0.2	−0.34	0.74	0.47
Intermediate Achenes					
D_1 (11.2% ± 7.1)	D_4 (32.1% ± 9.7)	−0.54	−1.12	0.04	0.07
D_1	D_7 (1.2% ± 0.94)	0.14	−0.34	0.68	0.57
D_1	D_{14} (2.1% ± 1.5)	0.09	−0.44	0.66	0.75
D_1	D_{21} (0% ± 0)	0.15	−0.36	0.75	0.59
D_4 (32.1% ± 9.7)	D_7 (1.2% ± 0.94)	0.67	0.12	1.21	0.02
D_4	D_{14} (2.1% ± 1.5)	0.63	0.05	1.67	0.03
D_4	D_{21} (0% ± 0)	0.68	0.16	1.29	0.01
D_7 (1.2% ± 0.94)	D_{14} (2.1% ± 1.5)	−0.05	−0.53	0.40	0.82
D_7	D_{21} (0% ± 0)	0.01	−0.45	0.48	0.95
D_{14} (2.1% ± 1.5)	D_{21} (0% ± 0)	0.06	−0.39	0.52	0.79
Ray Achenes					
D_1 (10.2% ± 6.6)	D_4 (20.4% ± 9.2)	−0.26	−0.83	0.33	0.37
D_1	D_7 (0.31% ± 0.31)	0.15	−0.34	0.66	0.56
D_1	D_{14} (0.72% ± 0.72)	0.12	−0.39	0.68	0.67
D_1	D_{21} (0% ± 0)	0.15	−0.38	0.67	0.57
D_4 (20.4% ± 9.2)	D_7 (0.31% ± 0.31)	0.41	−0.15	0.96	0.13
D_4	D_{14} (0.72% ± 0.72)	0.39	−0.21	0.93	0.17
D_4	D_{21} (0% ± 0)	0.42	−0.12	0.93	0.13
D_7 (0.31% ± 0.31)	D_{14} (0.72% ± 0.72)	−0.02	−0.47	0.44	0.92
D_7	D_{21} (0% ± 0)	0.0004	−0.45	0.46	0.99
D_{14} (0.72% ± 0.72)	D_{21} (0% ± 0)	0.03	−0.39	0.51	0.9

Notes: Mean consumption proportions for overall, disc, intermediate, and ray achene's seeds between treatments of artificial *Schinia mortua* (Noctuidae) larvae inoculation at 1 (D_1), 4 (D_4), 7 (D_7), 14 (D_{14}), and 21 (D_{21}) days after *Grindelia ciliata* (Asteraceae) capitulum opening. Displayed are the estimates of means of the posterior distributions (estimate) and their 95% credible intervals (CI). Significance is indicated by 95% CI that do not span 0 and a pMCMC less than 0.05.20

Chapter 3: Figures

Capitulum



Achenes

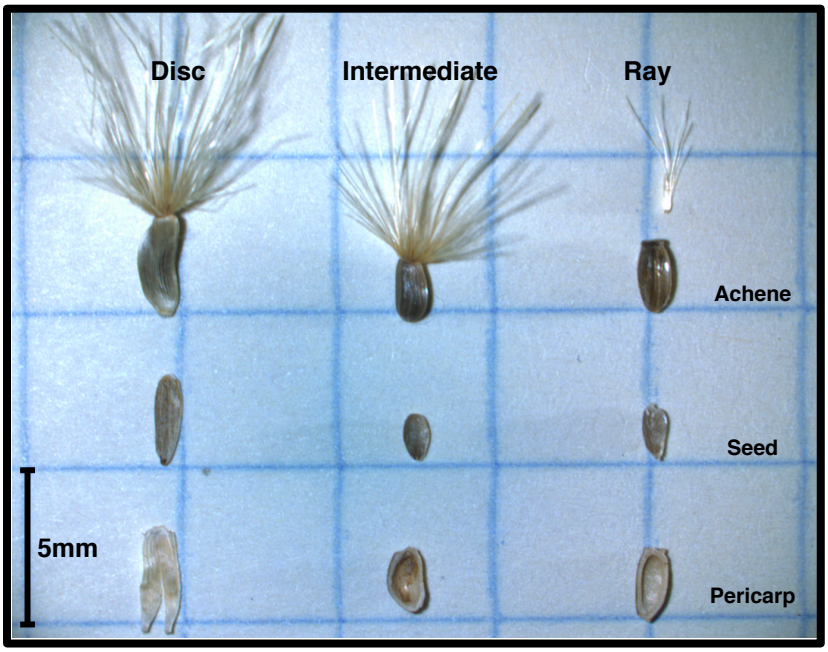


Figure 1. A longitudinal section of a *Grindelia ciliata* (Asteraceae) capitulum, showing the position of ray, intermediate, and disc achenes. Also shown are entire disc, intermediate and ray achenes, seeds, and longitudinal section of their pericarps.

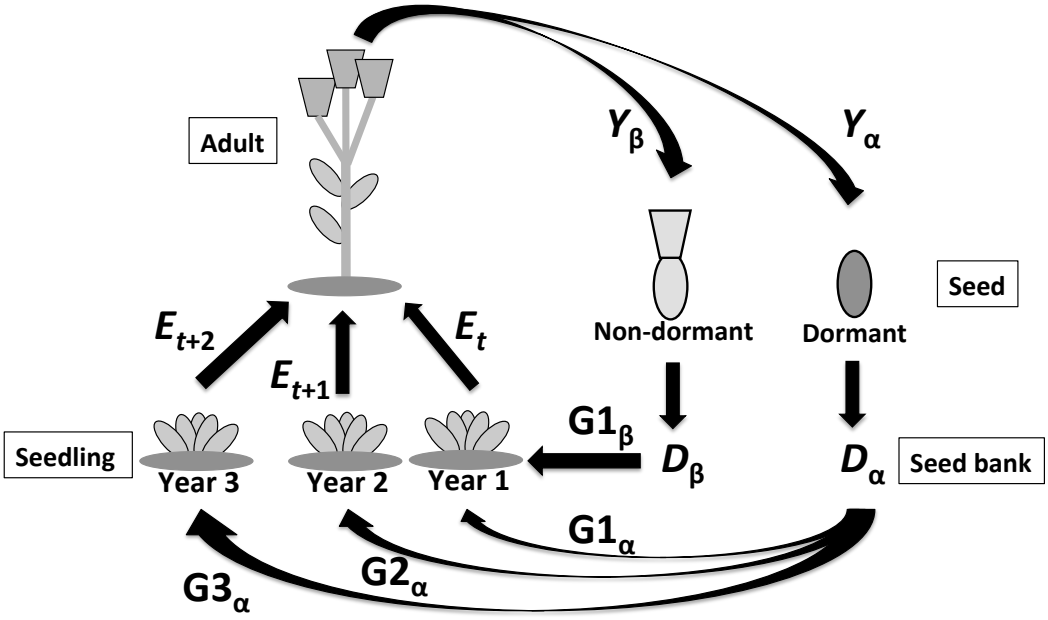


Figure 2. Diagram of the *Grindelia ciliata* (Asteraceae) life cycle (sporophyte phase). Arrows represent pathways through the life cycle in our models. Letters represent vital rates as described in Eq. 1. Adult production of dormant achenes is represented by Y_α, dormant achene mortality in the seed bank is represented by D_α, dormant achene seedling emergence in the first fall and spring after dispersal is $G1_\alpha$, dormant achene seedling emergence in the fall and spring one year after dispersal is $G2_\alpha$, dormant achene seedling emergence in the fall and spring two years after dispersal is $G3_\alpha$. Adult production of nondormant achenes is represented by Y_β, nondormant achene mortality in the seed bank is represented by D_β and nondormant achene seedling emergence in the first fall and spring after dispersal is $G1_\beta$. Seedling survival after germinating during the

year of dispersal is represented by E_{τ}, one year after dispersal by $E_{\tau+1}$, and during two years after dispersal by $E_{\tau+2}$.

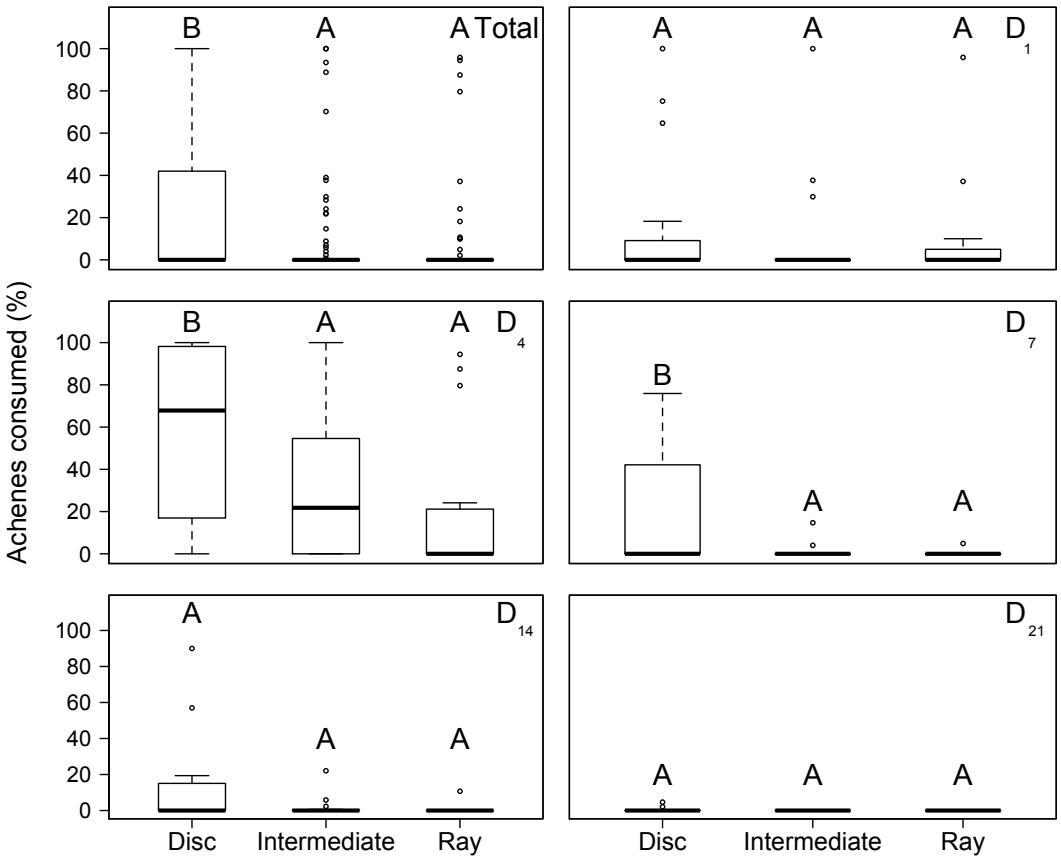


Figure 3. Box and whisker plot showing median (thick horizontal black line), Interquartile Range (upper and lower hinge), 25th and 75th percentiles (lower and upper whiskers), and outliers (open circles) of consumed *Grindelia ciliata* (Asteraceae) disc, intermediate, and ray achenes from all oviposited capitula (Total, $n = 78$), and from capitula with ovipositing on the 1st (D_1, $n = 15$), 4th (D_4, $n = 15$), 7th (D_7, $n = 16$), 14th (D_{14}, $n = 15$), and 21st (D_{21}, $n = 17$) day after capitulum opening. Differing letters indicate a significant difference among achene types ($p < 0.05$).

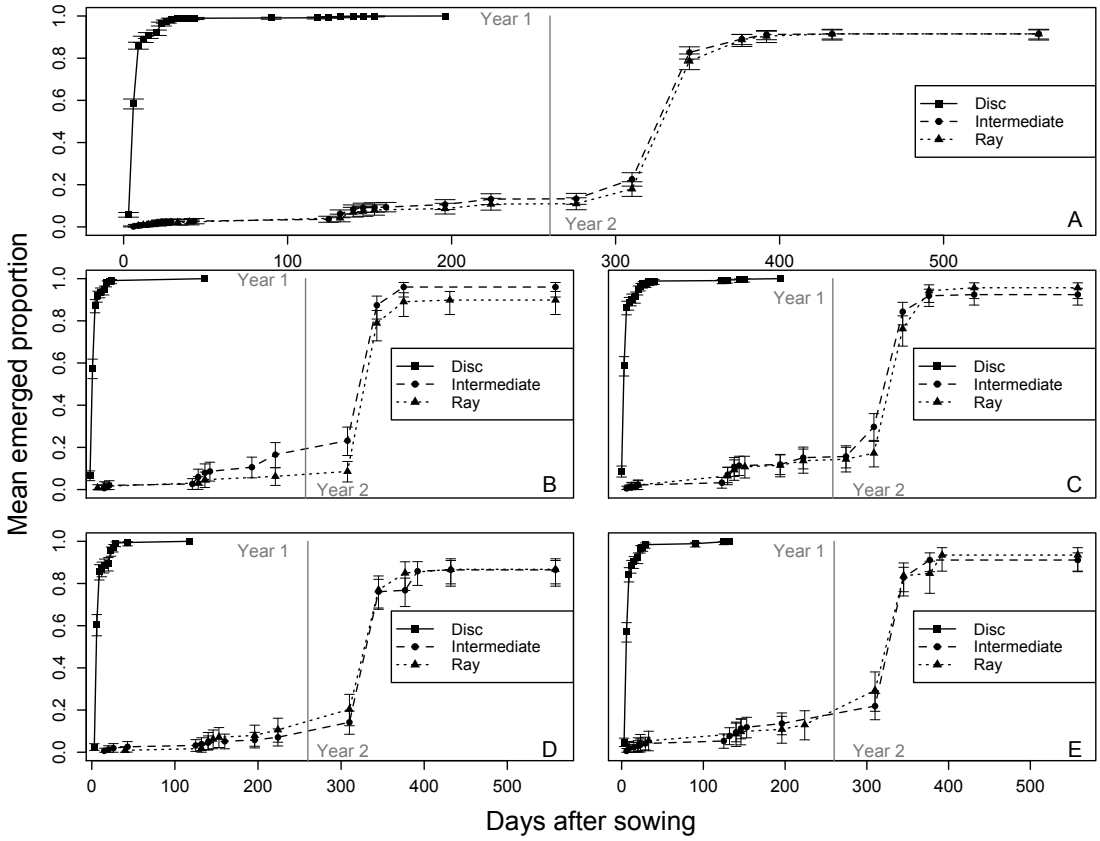


Figure 4. Mean field emergence proportion of *Grindelia ciliata* (Asteraceae) disc, intermediate, and ray achenes from (A) overall, (B) control, (C) 15-d stratified, (D) 30-d stratified, and (E) scarified storage treatments over two growing seasons (years). Error bar indicate 95% confidence intervals. Gray vertical line represents Heaviside cut point separating year 1 and year 2 seedling emergence cohorts.

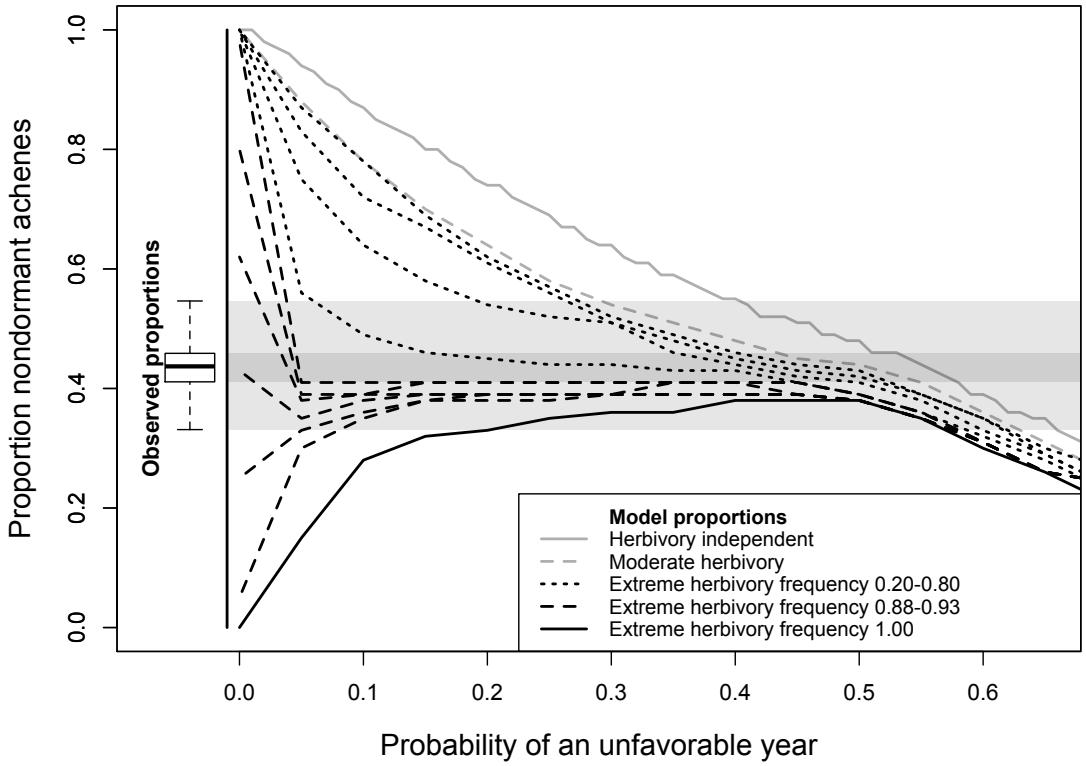


Figure 5. Deterministic model results show the relationship between nondormant achene allocation of optimal strategies in environments that differ in frequency of unfavorable years and the frequency of extreme herbivory. The solid gray line represents the optimal achene allocation proportions from the herbivory-independent model. The dashed gray line represents the moderate herbivory model, which lacks extreme herbivory events. The dotted black lines from top to bottom represent herbivory events with extreme frequencies of 0.20, 0.40, 0.60, and 0.80, respectively. The dashed black lines, from top to bottom, represent frequencies of 0.88, 0.89, 0.90, 0.91, 0.92, and 0.93 for extreme herbivory events. The solid black line represents constant extreme herbivory. Proportions of *Grindelia ciliata* (Asteraceae) nondormant

(disc) achenes produced by experimental plants in the larval herbivory experiment are shown by box plots with 25% quartile, median, 75% quartile (dark gray shade), and whiskers that extend to data extremes (light gray shade).

Chapter 4: Discussion

Although our theoretical understanding of bet-hedging in plants is thorough, our documentation of the selection pressures that shape plant bet-hedging strategies is in its adolescence. In this dissertation, I present the first evidence that pre-dispersal herbivory is a significant selection pressure that influences the evolution of the bet-hedging strategy heterocarpy in *Grindelia ciliata* (Asteraceae). Pre-dispersal herbivory is well documented as having significant impacts on plant fitness, however it was never shown to be a mechanism that influences the evolution of heterocarpy. Because pre-dispersal herbivory is common in most ecosystems, and therefore, likely influences species in a similar manner as was observed in *G. ciliata*, there may be great opportunities to learn, and consequently, great deficiencies in the understanding of bet-hedging in other species and ecosystems. Furthermore, if such a common and strong selection factor has not been considered before, what other factors might we be missing?

Seedbank mortality is another aspect that has not received thorough attention in most plant bet-hedging systems. Although it is well known that annual plants in particular can have long lived seedbanks, it is not clear how differences in propagule morphology and physiology affect survival in the seedbank. This lack of understanding is driven in part because seedbank studies are logistically difficult to conduct, especially when researchers are also interested in understanding when seeds will emerge from the seedbank naturally. Furthermore, the longevity of seedbank studies is a big limitation to our understanding of variability of mortality in the seedbank. For example, the seedbank mortality risks that are presented herein are representative for only one and a half years, which is not an adequate timespan to capture the variability in soil microbiome, soil

moisture, and soil temperature dynamics, and these are just a few contributing factors that may affect seedbank mortality.

The timing of seed germination and its effects on individual fitness may be the most well studied aspect of bet-hedging in plants. However, this selection pressure is perhaps the least understood in *G. ciliata*. There is a unique and powerful resource available, the Oklahoma Mesonet, for studying the relationship between soil moisture dynamics and seedling mortality across the native range of *G. ciliata* in Oklahoma. This creates an exceptional opportunity for developing an understanding of selection acting on the timing of germination in *G. ciliata*.

Appendix A: References to Abstract

Cohen D. 1966. Optimizing reproduction in a randomly varying environment. *Journal of Theoretical Biology* 12: 119 – 129.

Gillespie J. H. 1974 Natural selection for within generation variance in offspring number. *Genetics* 76: 601 – 606.

Gremer J. R. and D. L. Venable. 2014. Bet hedging in desert winter annual plants: optimal germination strategies in a variable environment. *Ecology Letters* 17 (3): 380-387

Kleinbaum D. G. and M. Klein. 2012. *Survival Analysis*. Springer Science Inc., New York.

Philippi T, J. Seger. 1989. Hedging ones evolutionary bets, revisited. *Trends Ecology and Evolution* 4: 41 – 44.

Ritz C., C. B. Pipper, J. C. Steibig. 2013. Analysis of germination data from agricultural experiments. *European Journal of Agronomy* 45, 1-6.

Seger J. and H. J. Brockmann. 1987. What is bet-hedging? *Oxford Surveys in Evolutionary Biology* 4: 182 – 211.

Simons A. M. 2011. Modes of response to environmental change and the elusive empirical evidence for bet hedging. *Proceedings of the Royal Society Biological Sciences* 278: 1601–1609.

Appendix B: Supplemental material to Chapter 1

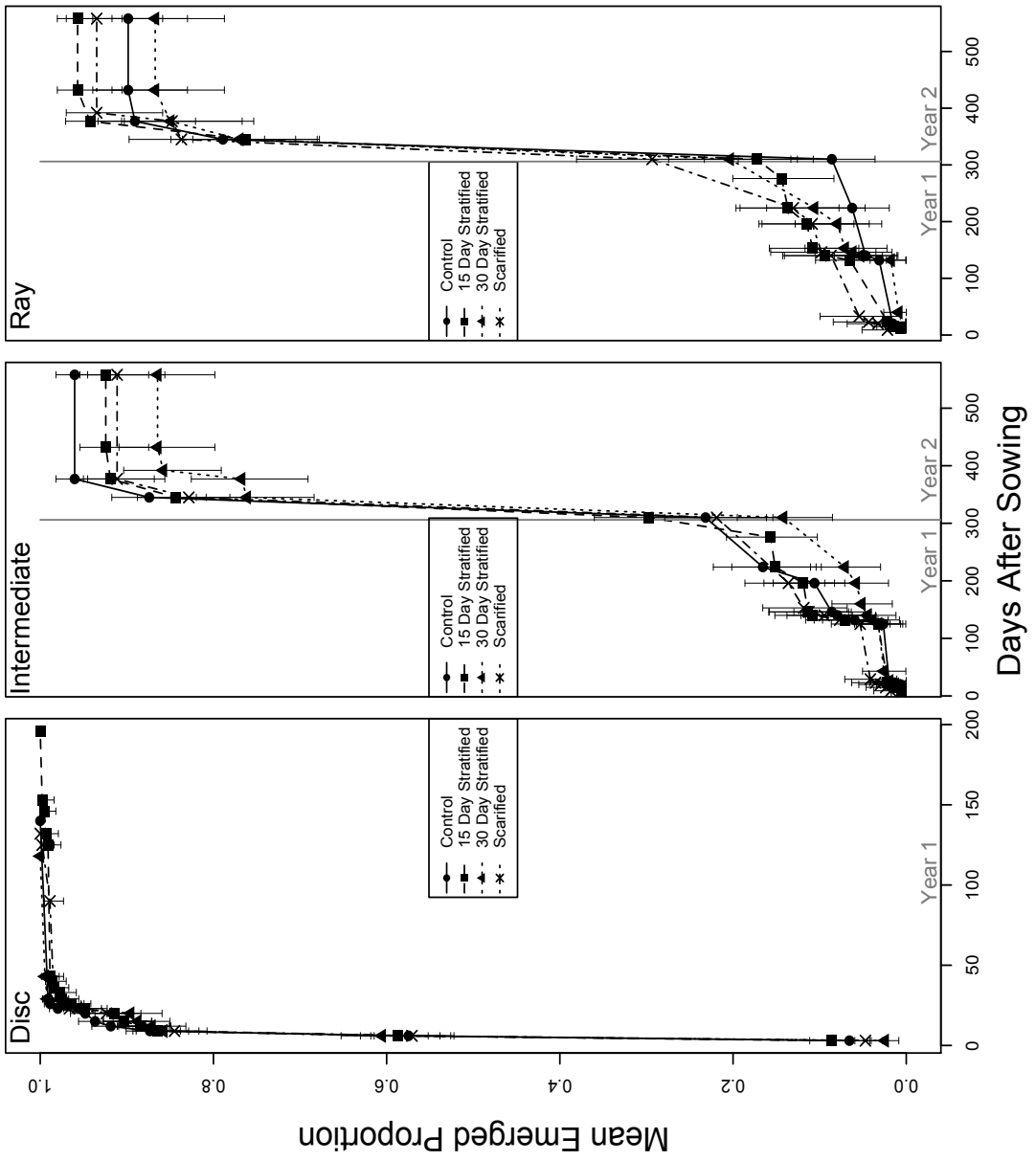
#Running the extended Cox Proportional Hazard model with two Heaviside functions.

#To alter Heaviside function intervals, users must modify the code containing the #*survSplit()* function, and the code thereafter. In *survSplit()*, the user must select other #time intervals at which to cut the dataset. Then, the user must create new Heaviside #objects in the new dataset created by *survSplit()*. For example, if the aim is to create #two Heaviside functions, one including days prior to Day2 = 20 and another for days #post Day2 = 20, then,

```
pops.cph30=survSplit(Surv(Day1,Day2,Event)~Ray,germ,cut=c(16),end="Day2",
event="Event",start="Day1")
pops.cph30$hvRpre=pops.cph30$Ray*(pops.cph30$Day1<16)
pops.cph30$hvRpost=pops.cph30$Ray*(pops.cph30$Day1>16)
pops.cph30[,7]<-germ[,1]
pops.cph30[,8]<-germ[,2]
colnames(pops.cph30)=c("Ray","Day1","Day2","Event","hvRpre","hvRpost","Individu
al","Rep")
Y30=Surv(pops.cph30$Day1,pops.cph30$Day2, pops.cph30$Event)
Cox.H2.SQ <-coxph(Y30 ~ hvRpre+hvRpost+cluster(Rep) +cluster(Individual),
data=pops.cph30)
 summary(Cox.H2.SQ)
```

Appendix C: Supplemental material to Chapter 3

Appendix B. Mean *Grindelia ciliata* (Asteraceae) seedling emergence proportions from disc, intermediate, and ray seedlings in the field over two years. Control (●), 15-day stratification (■), 30-day stratification(▲) and scarification (×) treatments and shown for each achene type. Error bars indicate 95% confidence intervals. Grey vertical line represents Heaviside cut point separating year 1 and year 2 seedling cohorts.


Disc
Intermediate
Ray
Control
15 Day Stratified
30 Day Stratified
Scarified
Year 1
Year 2
Mean Emerged Proportion
Days After Sowing
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200
300
400
500