Bet-hedging against larval herbivory and seed bank mortality in the evolution of heterocarpy[1]

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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.

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

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, b; 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, 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, 1982a).

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, personal observation). 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.

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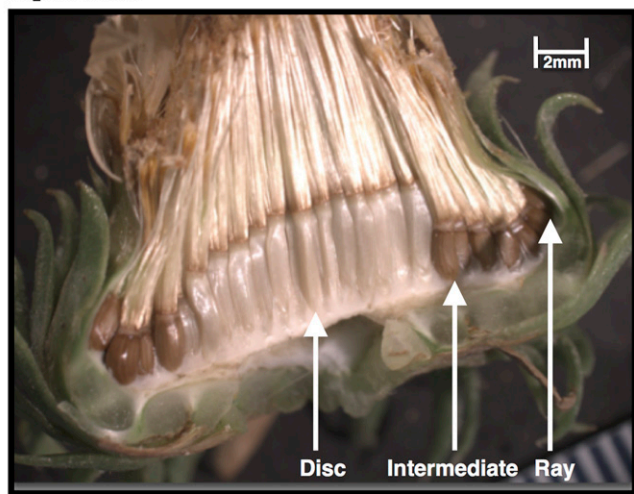
Achenes
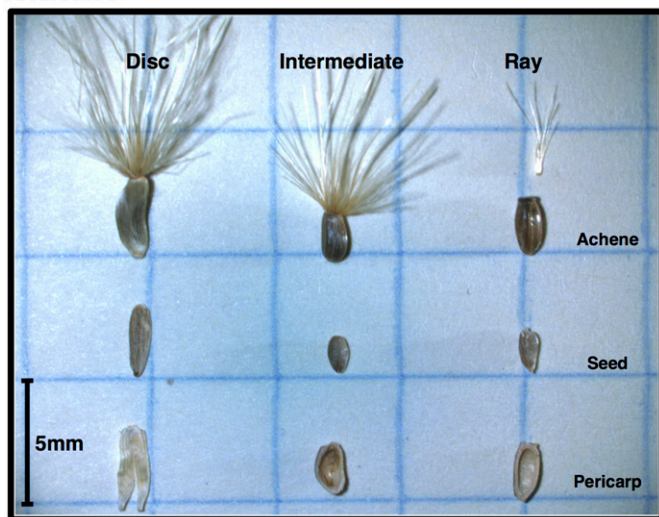
FIGURE 1 Longitudinal section of a *Grindelia ciliata* (Asteraceae) capitulum, showing the position of ray, intermediate, and disc achenes. Also shown are entire disc, intermediate and ray achenes, seeds, and longitudinal section of their pericarps.

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.

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; 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 [, 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 on 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 honestly significant difference (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 environ-

ments 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):

$$R_0 = \begin{bmatrix} \left[Y_\alpha(1-D_\alpha)G1_\alpha E_t\right] + \left[\left[Y_\alpha(1-D_\alpha)(1-G1_\alpha)\right]G2_\alpha E_{t+1}\right] \\ + \left[\left[Y_\alpha(1-D_\alpha)(1-G1_\alpha)(1-G2_\alpha)\right]E_{t+2}\right] \end{bmatrix} + \left[Y_\beta(1-D_\beta)G1_\beta E_t\right],$$

(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
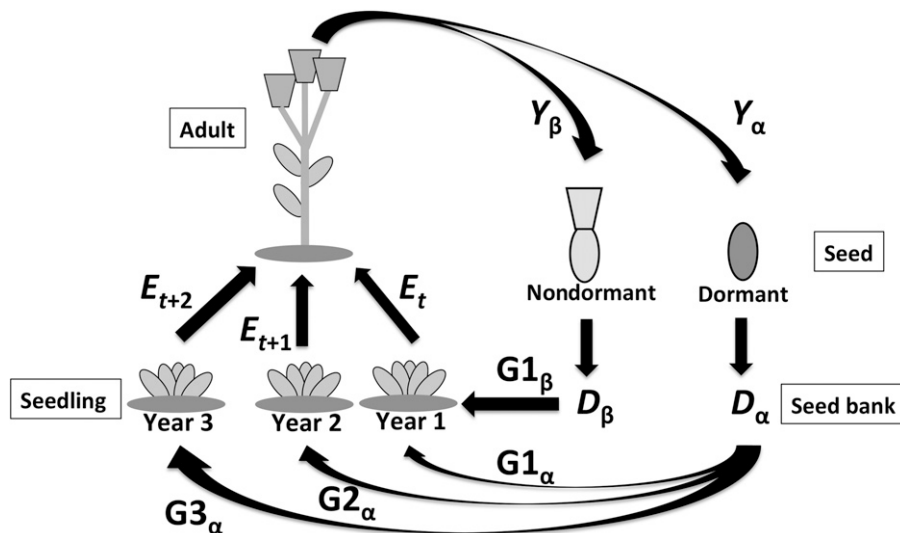
FIGURE 2 Diagram of the *Grindelia ciliata* (Asteraceae) life cycle (sporophyte phase). Arrows represent pathways through the life cycle in our models. Letters represent vital rates as described in Eq. 1. Adult production of dormant achenes is represented by Y_α, dormant achene mortality in the seed 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 1 year after dispersal is $G2_\alpha$, dormant achene seedling emergence in the fall and spring 2 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, 1 year after dispersal by $E_{\tau+1}$, and during 2 years after dispersal by $E_{\tau+2}$.

achenes relative to environmental quality by modifying Eq. 1 so that yield of dormant achenes after herbivory (YH_α) is

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

and the yield of nondormant achenes after herbivory (YH_β) is

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

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.

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.
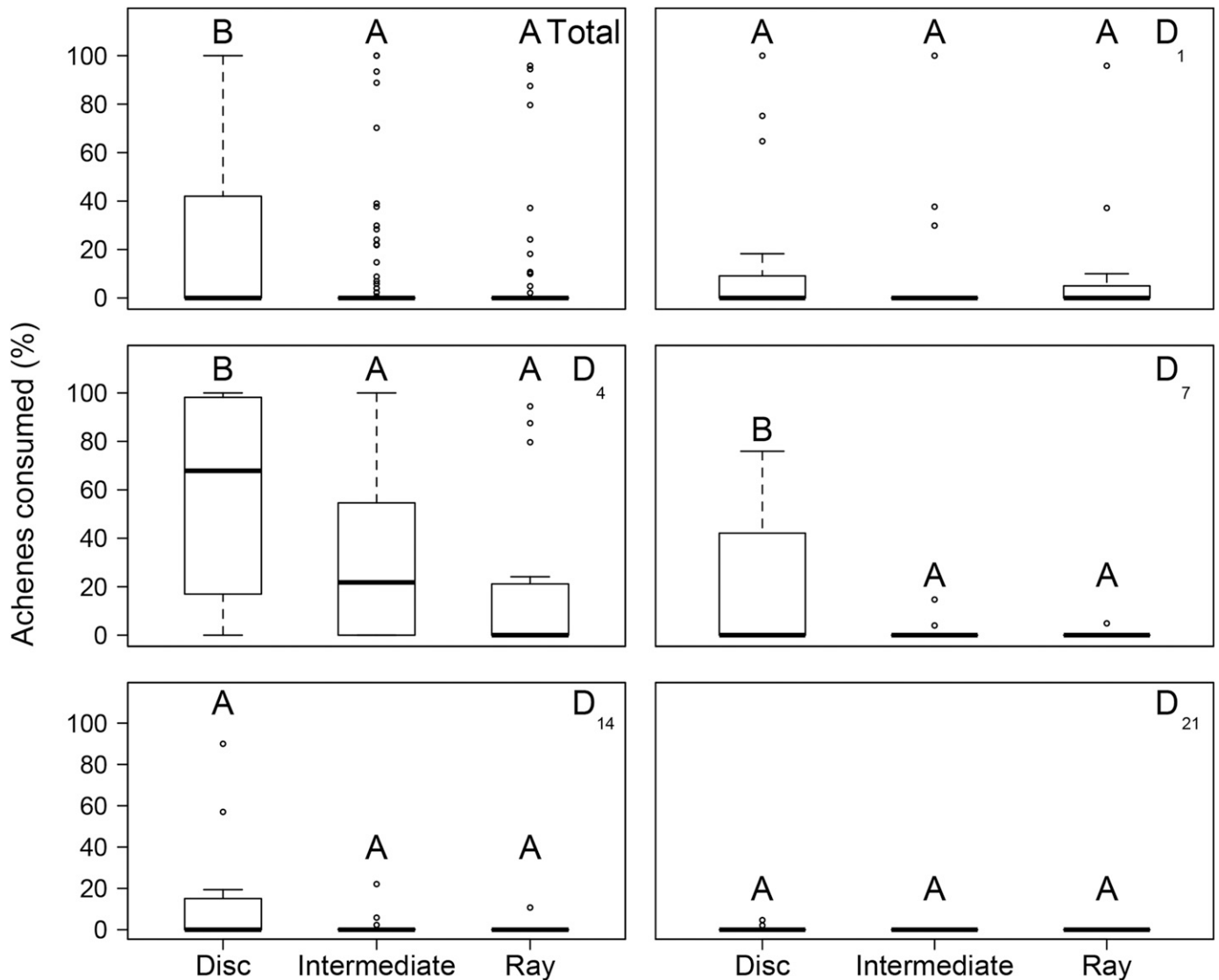
FIGURE 3 Box and whisker plot showing median (thick horizontal black line), interquartile range (upper and lower hinge), 25th and 75th percentiles (lower and upper whiskers), and outliers (open circles) of consumed *Grindelia ciliata* (Asteraceae) disc, intermediate, and ray achenes from all oviposited 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 ($p < 0.05$).

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

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 capitulum opening of *Grindelia ciliata* (Asteraceae). 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.

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 S1, see Supplemental Data with the online version of this article). Intermediate seedling emergence was 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 S1). 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 S1).

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 herbivory-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 herbivory-independent model (Fig. 5). The largest difference in achene allocation proportion was 1.0 (i.e., 100%),
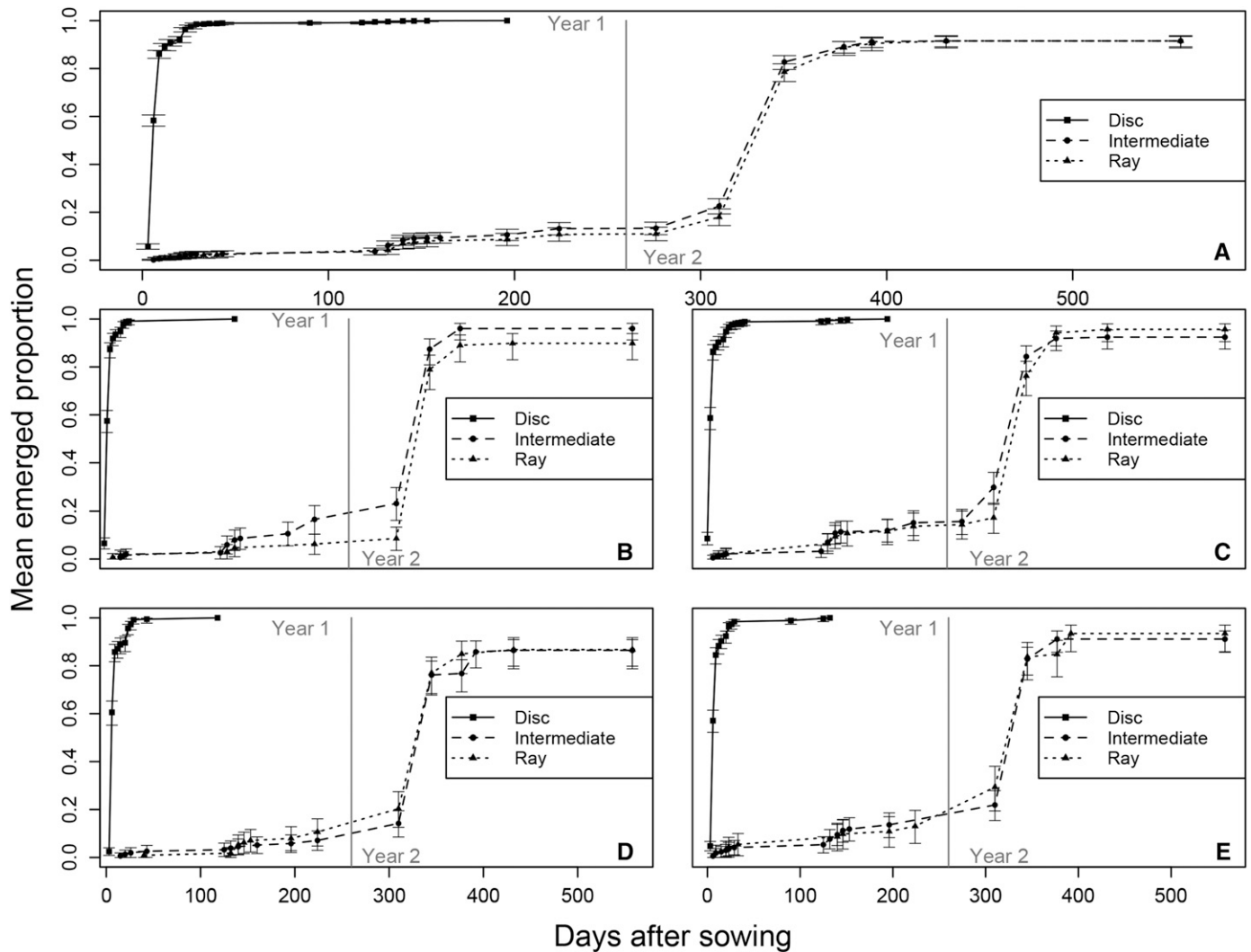
FIGURE 4 Mean field emergence proportion of *Grindelia ciliata* (Asteraceae) disc, intermediate, and ray achenes from (A) overall, (B) control, (C) 15-d stratified, (D) 30-d stratified, and (E) scarified storage treatments over two growing seasons (years). Error bar indicate 95% confidence intervals. Gray vertical line represents Heaviside cut point separating year 1 and year 2 seedling emergence cohorts.

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).

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
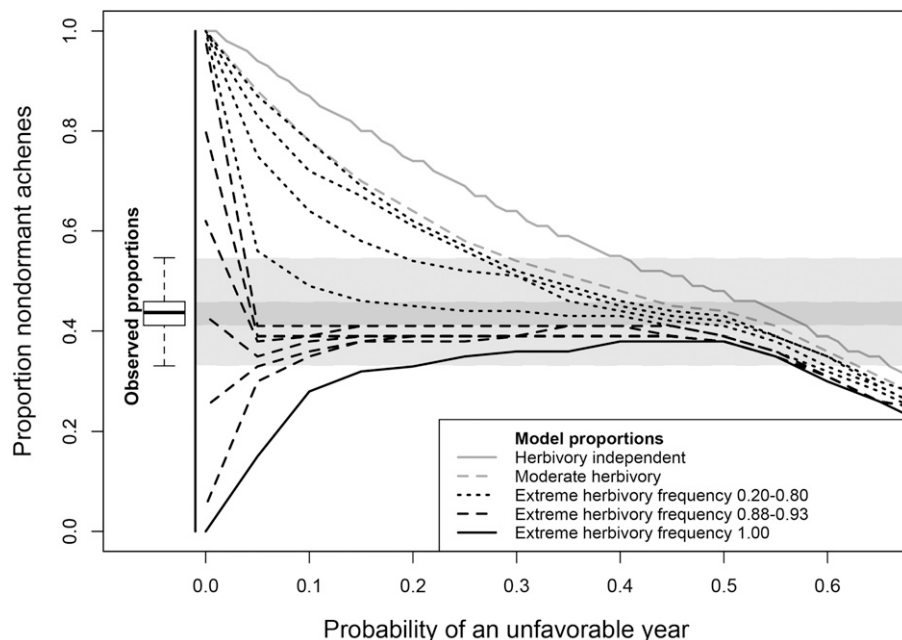
FIGURE 5 Deterministic model results show the relationship between nondormant achene allocation of optimal strategies in environments that differ in frequency of unfavorable years and the frequency of extreme herbivory. The solid gray line represents the optimal achene allocation proportions from the 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).

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 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 S1). 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; Rose et al., 2009; Shefferson, 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 from insect herbivory, it is likely a significant evolutionary pressure affecting reproductive bet-hedging not only in heteromorphic species but in 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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