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Plant Responses to Joint Effects of Herbivores and Pollinators

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PLANT RESPONSES TO JOINT EFFECTS OF HERBIVORES AND POLLINATORS

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ABSTRACT

Plants are fed upon by a range of insect foragers, including herbivores and pollinators. Because herbivores damage plant parts and pollinators transfer pollen among plants, plants generally benefit by avoiding herbivores and attracting pollinators. Through interactions with their host plants, herbivores and pollinators can influence the expression and evolution of plant traits. While there is a substantial body of research on plant-herbivore and plant-pollinator interactions, and increasing appreciation for the joint effects of herbivores and pollinators on plants, there are still aspects of plant-pollinator-herbivore interactions that warrant attention, including the persistence of effects beyond the year in which the interaction occurred, the effects of variability in herbivory and pollination on the evolution of plant traits, and the effects of herbivores and pollinators on asexual reproduction in plants.

Ecological interactions between foragers (pollinators and herbivores) and host plants make possible a network of feedbacks in which foragers both influence and respond to plant traits. These feedbacks can link the plant traits to which foragers respond, and thus may help explain observed variation in plant traits. Interactions among plants, herbivores, and pollinators have been well documented, but addressing interactions among all three across years in a single system is rare. Across year effects are particularly important because single-year studies might misinterpret plant responses to their environment. In chapter two I describe two experiments using the perennial plant *Chamerion angustifolium* that address plant-forager interactions. In one I manipulated herbivory and pollen receipt to quantify forager effects on plant traits and in another I manipulated plant size and flowering phenotype to quantify forager response. I found pathways of interaction between plants and insects both within and across years, suggesting the potential for feedback between foragers and plant traits. Results suggest that while pollinators prefer plants with more flowers, and pollen receipt results in smaller plants, herbivores cause size overcompensation and flower reduction. Together these effects of both herbivores and pollinators may help maintain intermediate values of size and flowering traits.

Environmental conditions can have a profound influence on plant fitness, and can vary substantially in time. When environmental variability is unpredictable, that is, when plants have no cues as to upcoming environmental conditions, they should evolve a bet-hedging strategy to deal with environmental variability. In chapter three, I constructed a simulation model to address the evolution of the timing and pattern of resource allocation (allocation schedules) in annual and perennial plants under stochastic variability in herbivory and pollination. Both herbivory and pollination can be highly variable in space and time, but we don't fully understand how this variability influences the evolution of plant traits. I found that annual plants flower later in the growing season and perennial plants have early and prolonged flowering under high environmental variability. I found that across-year variability selected for late flowering in annuals and for early and prolonged flowering in perennials, suggesting that populations of annuals and perennials should evolve different types of allocation schedules under variable herbivory and pollination.

The biotic environment plays an important role in whether a species will be able to invade a new habitat. Herbivores and pollinators may influence the spread of plant species by influencing allocation to reproduction. Many plants reproduce both sexually and asexually, and the mode of reproduction a plant expresses can influence colonization of and establishment in new habitats. In chapter four, , I describe a series of studies using *Eichhornia crassipes* (water hyacinth) to examine the effects of pollination and simulated (manual) larval and adult herbivore

damage on plant growth, sexual and asexual reproduction, and herbivore resistance. Herbivores and pollinators are known to influence a wide variety of plant traits, but despite the importance of mode of reproduction for plant population dynamics, we know little about how clonal plants respond reproductively to herbivory or pollination. I conducted surveys of natural populations to assess differences in allocation pattern, herbivore damage, and herbivore resistance, and used a common garden experiment to determine if differences in allocation pattern or resistance are due to environment or among-population genotypic differences. I found that the damage mimicking larval feeding generally shifted plant responses toward asexual reproduction, while adult-type damage and pollination had no effect, demonstrating that plant foragers and forager identity can have important, but as yet incompletely understood, effects on asexual plant reproduction.

Although there is a large body of research on plant-herbivore and plant-pollinator interactions, there remain complexities in these interactions that warrant further research. Results from my dissertation demonstrate that (1) multi-year studies on perennial plants are necessary to understand the effects of foragers on perennial plants, (2) forager variability might have important effects on the evolution of plant traits, and (3) foragers can have important effects on plant asexual reproduction.

CHAPTER ONE

INTRODUCTION

Plants and plant-feeding insects together comprise half of known macroscopic species (Bernays and Chapman, 1994). Many insects depend on plant hosts for food, sometimes spending their entire life cycle on a single host plant species (for example, the senita moth *Upiga virescens* on senita cactus *Pachycereus schottii*, or the false potato beetle *Leptinotarsa juncta* on Carolina horsenettle *Solanum carolinense*). All plant parts can in turn be fed upon at any life stage, and insects may feed on any plant part. Insects foraging on plants can alter plant phenotype (Agrawal, 2001), population dynamics (Crawley, 1989), and evolution (Strauss and Agrawal, 1999). Although herbivores are commonly recognized as plant foragers, pollinators also forage at host plants, and can also alter plant phenotype (Ladio and Aizen, 1999; Clark and Husband, 2007) and evolution (Galen, 1989). Understanding interactions between insect forager behavior and the expression and evolution of plant traits is important for explaining the variation in plant traits we see in nature, how the biotic environment shapes that variation, and how host plants mediate forager interactions.

Flowering plants making use of insects as pollination vectors must invest in pollinator attraction and reward, offering large floral displays (Conner and Rush, 1996), scent (Andrews *et al.*, 2007), or nectar (Cresswell, 1999) to entice pollinators, but attractive traits may also be detected by antagonists including seed predators (Juenger *et al.*, 2005) and herbivores (Miller *et al.*, 2008). Because plants attract insect foragers but are influenced by them as well, plants can mediate indirect interactions between foragers. For example, pollinator visitation can decrease following herbivory (Lehtilä and Strauss, 1997; Steets and Ashman, 2004). Insect foragers can therefore alter phenotypic expression of plant traits and act as agents of selection; these joint effects might prevent the evolution or expression of plant trait values that would optimize attractiveness to pollinators and avoidance of herbivores.

Across-year effects of herbivores and pollinators (from one growing season to the next) might cause plant traits to be poorly matched to current forager environments. For this reason, single-year studies of perennial plants (which are common) can misinterpret the cause of ecological variation in plant traits (Knight *et al.*, 2006). Quantifying plant-forager interactions both within and across years should help to more fully explain variation in plant size and flowering traits among years or locations. Multi-year studies of perennial plants are essential not only to assess across-year effects but also to capture the effects of temporal variability in herbivory and pollination. Herbivory and pollination are known to vary in both time and space (Huntly, 1991; Price *et al.*, 2005), and this variance have important effects on lifetime plant fitness and thus the evolution of plant traits (Via and Lande, 1985; Moran, 1992). Measuring lifetime patterns in long-lived plants, however, is quite challenging. Optimality models offer a way to predict the evolution of plant traits in complex environments, and have given us expectations for how annual and perennial plants should evolve when the environment does not vary (Cohen, 1971), and when it does (King and Roughgarden, 1982; Wong and Ackerly, 2005). While previous models do address stochastic variability in season length (when plants die due to the end of the season, for example by frost; hereafter "end-of-season") or disturbance, models have not yet looked at antagonistic effects (like herbivory) and mutualistic effects (like pollination) simultaneously varying across time. Because herbivores and pollinators both interact with plants, including them both in models predicting plant trait evolution is important.

Plant size and flowering responses are frequently the focus of host plant-forager research. These traits can be important for plant performance and fitness, but many plants reproduce asexually as well as sexually. Clonal plants (those which do some asexual reproduction) are common, and mode of reproduction for clonal plants can have important implications for plant population spread through dispersal ability and offspring establishment rate (Eriksson, 1997; Herrera and Nassar, 2009). The effect of herbivores and pollinators on plant mode of reproduction could therefore inform our understanding of plant population responses to herbivory and pollination, and would be especially important for predicting how invasive plant populations might respond to herbivores and pollinators in a new range. We still know little about clonal plant responses to herbivory and pollination, but some research suggests that sexual reproduction may be compromised by herbivory while asexual reproduction is not (Meyer and Root, 1993; Bråthen and Junttila, 2006; Wise *et al.*, 2006). If herbivory or pollination can alter investment in both modes of reproduction they need to be taken into account in trying to understand causes of variation in plant traits.

In this dissertation I describe field experiments and a mathematical model addressing the influence of insect herbivores and pollinators on the expression and evolution of plant traits. In the study described in chapter two, I manipulated herbivory, pollination and plant flowering and size traits in *Chamerion angustifolium* to investigate within- and across-year relationships between foragers and their host plants. I address how traits important for plant performance respond to changes in herbivory and pollination, how herbivore and pollinator behavior are influenced by plant traits, and how plants mediate indirect interactions between herbivores and pollinators, both within a single season and in the season following manipulations. In the study described in chapter three, I develop a stochastic simulation model that determines fitness outcomes for annual and perennial plants across a range of allocation schedules to growth, reproduction, and storage under different levels of variability in herbivory and pollination. With this model I address how biotic environmental variability interacts with allocation timing schedules to influence selection on plant traits. In the study described in chapter four, I manipulated damage and pollination received by *Eichhornia crassipes* and measured plant allocation to growth, sexual reproduction, asexual reproduction, and resistance. With these experiments I address how amount and type of damage, as well as pollination, affect mode of reproduction in a clonal invasive flowering plant. As a whole, this dissertation informs our understanding of how insect herbivores and pollinators interact with plants to create variation in plant traits, and how plant traits mediate interactions between herbivores and pollinators.

CHAPTER TWO

PLANT-FORAGER INTERACTIONS WITHIN AND ACROSS YEARS

INTRODUCTION

Many terrestrial flowering plants must both attract pollinators and avoid herbivores. This is a challenge because ecological interactions with both of these types of foragers can link the plant traits to which these foragers respond. For example, larger plant size or flower size may attract the beneficial services of pollinators but may also make a plant more apparent to herbivores, and therefore at a greater risk for damage (e.g., Miller *et al.*, 2008). It is known that both types of foragers respond to plant size and flowering traits: herbivores and pollinators can be attracted to taller plants (Carromero and Hamrick, 2005; Juenger *et al.*, 2005) and to plants with larger floral displays (Miller *et al.*, 2008). Considering the simultaneous effects of antagonists and mutualists can reveal patterns that considering just one group alone will not reveal (Lehtilä and Strauss, 1997; Steets and Ashman, 2004; Strauss and Murch, 2004). The joint effects of antagonists and mutualists are perhaps most clear when a single insect species acts in both roles, for example in pollinating moths that lay eggs on host plants, such as the senita moth *Upiga virescens* on senita cactus *Pachycereus schottii* or the hawk moth *Manduca sexta* on tobacco *Nicotiana attenuata*. *Nicotiana attenuata* has been shown to alter its flowering time in response to *M. sexta* damage in order to attract a different pollinator species (Kessler *et al.*, 2010).

Traits of individual plants are also known to change in response to damage or pollination. Herbivores generally have negative effects on traits like height (Schat and Blossey, 2005), flower number (Quesada *et al.*, 1995), flower size (Steets and Ashman, 2004), and fruit number (Wise and Sacchi, 1996). However, overcompensation, or increased growth or reproduction in response to herbivory, has also been demonstrated (Paige and Whitham, 1987; Gadd *et al.*, 2001). Pollen receipt can reduce the number of open flowers (Clark and Husband, 2007) and subsequent nectar production (Ladio and Aizen, 1999), and also has well-recognized positive effects on seed set (Campbell and Halama, 1993).

Because foragers both influence and respond to plant traits, they can influence one another indirectly via plant traits. Damage has been frequently shown to decrease pollinator attraction by reducing flower number or size (Lehtilä and Strauss, 1997; Steets and Ashman, 2004), making herbivores doubly detrimental to plants as they both consume plant parts and reduce attractiveness to pollinators. Positive indirect effects of pollinators on fruit-eating mammalian herbivores have been found (Herrera, 2000), but to our knowledge effects of pollinators on leaf herbivores remain unexplored.

Just as indirect effects might complicate interpretation of forager effects on plant traits, so might correlations between different plant traits. In most cases where a relationship between plant size and flowering has been found, size correlates positively with flowering (e.g., Herrera, 2004), perhaps because size influences photosynthetic capabilities and the size of the resource pool. Thus feedback between plant traits and forager behavior and correlations among plant traits provide a network of pathways by which foragers might influence variation in size and flowering traits both in the short term (e.g., differences in trait expression among environments) and long term (e.g., evolution of plant traits). While most potential pathways for ecological feedbacks between herbivores, pollinators and plant size and flowering traits within a single growing

season have been well explored, studies have rarely considered more than a few of these pathways at once (but see Strauss *et al.*, 1999; Miller *et al.*, 2008). Thus there is still much to explore about how many of these pathways are likely to co-occur and interact within a given system.

If interactions with pollinators, herbivores, or both induce plant responses in the year following the interaction, plant traits might be poorly matched to their current forager environment, and single-year studies may misinterpret the cause of ecological variation in perennial plant traits. Short-term studies, for example, may overestimate effect size of pollen limitation (Knight *et al.*, 2006). While measuring lifetime traits in perennial plants is challenging, single-season studies of perennial plants are common. For perennial plants, some pathways of effects may even be more likely to occur across, rather than within, years. Pollinators, for example, generally interact with plants later in the growing season relative to leaf herbivores, which are typically present in some form throughout the growing season. This means that pollinators may be less likely to strongly influence the behavior of herbivores by changing plant traits within a season, but investment in fruit following pollination may alter traits in later seasons (e.g., Lehtila and Syrjanen, 1995). While it is thus plausible that indirect effects between forager types might occur in one direction within years, and in another direction across years (e.g., herbivores might affect pollinators within years, but pollinators might affect herbivores across years), cross-season effects of pollinators on herbivores have not been explored, perhaps because most studies use annual plants for which across-year effects could only occur through seeds.

Quantifying interactions between foragers and plant traits should help to explain variation in plant size and flowering traits among years or locations. Because size and flowering can also be closely related to fitness, these forager effects may also affect plant population dynamics and the evolution of plant traits. For example, studies suggest that that herbivores and pollinators can respond similarly to plant traits (Miller *et al.*, 2008) but may exert opposing effects on fitness (Strauss *et al.*, 1999). In this study I focus on ecological interactions of insect foragers with plant traits, considering multiple pathways both within and across years. I examined interactions among herbivores, pollinators, plant size, and flowering in two separate experiments. I asked three specific questions:

(1) Do herbivores and pollinators respond to plant size and flowering phenotype?
(2) Do herbivore damage and pollination affect size and flowering traits?
(3) Are there correlations between size and flowering traits which could provide another pathway through which herbivore and pollinator effects might act?

Based on previous studies, I expect that herbivores and pollinators may respond similarly to plant traits. Since herbivores and pollinators have been found to have both positive and negative effects on plant traits in other systems, I cannot predict their specific effects here, but results from these experiments will reveal how herbivores and pollinators together might limit plant trait expression. I expect that size and flowering traits may correlate positively in this system, since larger plants should be able to produce more flowers. This would make potential conflicting effects of herbivores and pollinators on plant traits, not trade-offs between plant traits, the main drivers of constraints on the expression of plant traits .

MATERIALS AND METHODS

Study system

I conducted this research in the spring and summer of 2009 and 2010, at the Rocky Mountain Biological Laboratory (RMBL, Gunnison County, CO [N38.9398°, W106.9821°]) in natural populations of *Chamerion angustifolium*. Because it is a perennial with iteroparous sexual reproduction, relationships among *C. angustifolium* flowering, size, and foragers are possible both within and across years. *Chamerion angustifolium* produces many (hundreds per fruit) small wind-dispersed seeds as well as clonal stems from underground runners. Aboveground stems can be found singly or in clumps comprising one individual arising from the same rhizome. Each year all, some, or none of the stems of a given individual may produce a multi-flowered inflorescence, and while flowers are self-compatible, selfing rates are low (Myerscough, 1980; Husband and Schemske, 1997). Inbreeding depression in *C. angustifolium* is strong (Husband and Schemske, 1997), so outcrossing provided by pollinator services is important. *Chamerion angustifolium* at RMBL has several bumblebee pollinator species, primarily *Bombus flavifrons* and *B. bifarius*, as well as both specialist and generalist leaf herbivores including caterpillars (*Hyles lineata* and *H. gallii*) and adult chrysomelid beetles (*Bromius obscurus*). Mule deer (*Odocoileus hemionus*) are frequent *C. angustifolium* herbivores, browsing the top several centimeters of stem and inflorescences.

Herbivore and pollinator response to plant size and flowering phenotype

Experimental design

To determine the effects of plant size and flowering traits on pollination success and herbivore attack, I characterized forager responses to stem and flower removal, and measured fruit production (pollinator response) and damage (herbivore response) in the same year that treatments were imposed. Forager responses to phenotype alteration only make sense in the year of treatment, since forager behavior in subsequent years would be a response to changes in plant allocation pattern provoked by biomass removal, rather than the manipulated phenotype. Regardless, I measured plant size and flowering responses across years and saw no across-year effects of removal manipulations (data not shown, all $P > 0.3$), and so do not consider across year forager responses further. Herbivore responses were measured by proxy (i.e., percent damage) rather than by observing herbivore behavior directly (i.e., herbivore abundance). Damage can be considered a plant response as well as an herbivore response, and is therefore not a perfect measure of herbivory, I used this proxy because many insect herbivores on *C. angustifolium* forage at night or drop off the plant when disturbed. Likewise, pollinator responses were measured by proxy (i.e., fruit number) and fruit number can also be considered a plant response as well as a pollinator response, I use it because sufficient pollinator observations for an adequate sample size were not logistically possible.

In June 2009 I identified 105 *C. angustifolium* individuals in a single meadow; each individual had at least 10 stems and was approximately 20-30 cm in height. Individual plants were distinguished as a clump of stems separate from surrounding clumps by approximately 20 cm or more. I used a spade to trench to a depth of 20-25 cm in a perimeter giving an approximately 10-cm buffer around each individual to sever underground connections and reduce the influence of vegetatively associated, but unmanipulated, ramets. I assigned each individual to stem removal treatment, flower removal treatment, or control (no removal) treatment randomly with respect to plant position in the meadow, but re-assigned plants when

treatments were spatially clumped. Stem removal consisted of removing 50% of the stems at ground level with gardening shears; this treatment was imposed one time only in late June, after plants were approximately 30 cm tall on average. Flower removal consisted of removing 50% of the flowers and large buds at the stem with small scissors; this treatment was ongoing as flowers matured in August. Control treatment consisted of only handling plants.

I collected data every two weeks from June to August 2009 for a total of six surveys. I estimated average plant height over all stems from ground to stem tip (including inflorescences when present) using a meter stick centered within the stems. This method produced equivalent results to averaging individual measurements of each stem (A. Buchanan unpublished data). I visually estimated total percent damage (herbivore response) as leaf area missing per plant. Again, this method produced equivalent results to averaging per-leaf damage estimates for all leaves (A. Buchanan unpublished data). This gives an estimate for leaf chewing damage, but does not give an estimate for removal of entire leaves. Because leaves are produces at regular intervals and I did not notice substantial gaps in leaf formation, I believe that removal of entire leaves by insect herbivores was rare. Stem number, flower number, flowering stems, browsed stems, and fruit number (pollinator response) were counted directly.

Analysis

The effects of treatment on plant traits and proxies for forager responses were analyzed with one-way Type III SS analysis of variance (ANOVA) or generalized linear models (GLM). Because the data were unbalanced and we were not specifically interested in the order in which terms entered the models, we chose to use type III SS and interpreted main effects appropriately. Log transformed stem number, height, flower number (analyzed for flowering plants only) and log transformed percent damage were analyzed with ANOVA, with treatment as the independent fixed-factor variable. Transformations were made so that the data met assumptions of normality of residuals. Fruit number, which was analyzed for flowering plants only and with flower number as a covariate; number of browsed stems, with total stem number as a covariate; and number of flowering stems, with stem number as a covariate, were analyzed with GLM. Models were checked for possible overdispersion, which was negligible. Covariates in GLM models were retained in the model regardless of whether they significantly improved model fit because they were an essential part of the biological question. I used contrasts to test each treatment versus the control, but occasionally the data indicated that there may be a treatment versus non-treatment (control plus the other treatment) effect. In those cases, responses were analyzed again, grouping by treatment versus non-treatment. This second analysis was performed on browsed stems (within year response) and flower number (within year response). Of the nine total models, the final models with significant treatment effects are reported in Table 1; non-significant models are noted in the text. All analyses were performed in R 2.13.0 (R Development Core Team). ANOVA analyses used the package "car" (Fox and Weisberg, 2011).

Effect of herbivore damage and pollination on flowering and size traits

Experimental design

To determine the effects of herbivory and pollen receipt on plant size and flowering, I crossed two damage treatments (natural damage and reduced damage) with two pollination treatments (natural pollination and reduced pollination) for four total treatment groups. In June 2009 I identified 124 individuals in a meadow near RMBL that was separated from the previous experiment by approximately 0.5 km of aspen stands. I trenched around individuals using the methods described above. Carbaryl insecticide spray (Sevin® by GardenTech™, 22.5%

concentrate diluted to 5.85mL/L water) was applied to reduce leaf chewing herbivory, with water spray as a control. All plants were sprayed every two weeks until flowering began, for a total of three applications. Plants were not sprayed after flowering to prevent negative effects of insecticide on pollinators. Therefore some damage accumulated after insecticide treatment was stopped (Figure 1), but cumulative damage levels in insecticide-treated plants remained substantially lower than in control plants. I excised stigmas on most flowers per plant to prevent pollen receipt in the reduced pollination treatment. This technique has been used to prevent pollination in other systems (e.g., Ladio and Aizen, 1999), but can reduce flower lifespan in some species (e.g., Lovell *et al.*, 1987). Although I did not measure floral life span directly, I noticed no obvious reduction in flower lifespan following stigma excision. I chose this method instead of pollinator exclusion bags in order to minimize potential microclimatic effects (Kearns and Inouye, 1993) and to allow natural pollen and nectar removal from plants. I observed that greenhouse-reared *C. angustifolium* with no access to pollinators developed a sticky buildup of pollen and nectar in and around the flowers that might have promoted mold. Loss of plant biomass from stigma excision is likely negligible, and mechanical removal of plant parts often does not elicit the same defense response as real herbivory (Walling, 2000; Massey *et al.*, 2007).

Stigmas were removed from all or nearly all flowers on half the study plants every two to three days as new flowers opened. Control plants were handled but stigmas were left intact. *Chamerion angustifolium* flowers are protandrous and stigmas are not receptive until a few days after flowers open (Clark and Husband, 2007), so stigma removal within three days of flower opening should prevent pollination. For a one-week period in August, I were unable to access the plants, and although I isolated inflorescences with mesh pollinator exclusion bags, loss of some bags to wind during this period may have reduced the efficacy of pollination treatments. I measured plant responses (size and flowering traits) and forager responses (fruit set, deer browsing, and leaf damage by insects) as described in the previous experiment and on the same schedule. Treatments were imposed in 2009 only, while responses were measured in the treatment year (2009) and the following year (2010).

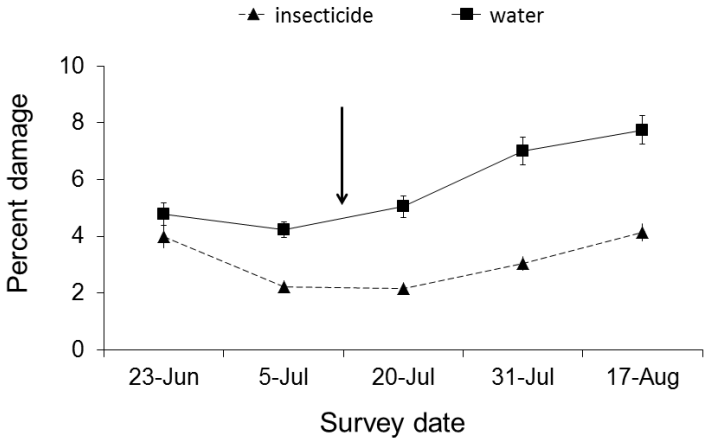

Figure 1. Insecticide decreases percent damage per plant. Damage levels rise slightly after the final insecticide application (July 13, 2009; indicated by arrow) but remain lower than water sprayed plants. Error bars show ± 1 SE.

Analysis

I analyzed treatment effects on plant traits and proxies for forager responses with either ANOVA or GLM, and analyzed all responses from both the treatment year and the following year. Stem number (log transformed in 2009, square-root transformed in 2010), height, flower number (which was analyzed for flowering plants only), and log transformed percent damage were analyzed in response to main and interactive effects of insecticide and stigma excision with Type III SS ANOVA; data met assumptions for ANOVA analysis after transformations resulted in approximately normal distributions of residuals. Fruit number (analyzed for flowering plants only) with flower number as covariate, number of browsed stems with stem number as covariate, and number of flowering stems with stem number as covariate were each analyzed in response to main and interactive effects of insecticide and stigma excision with GLM with negative binomial error distributions. Flower number and stem number covariates were included to account for effects of flower number on fruit number and effects of stem number on both number of browsed stems and number of flowering stems, and remained in the final GLM regardless of how they affected the fit of the final model. To test for excision effects, including insecticide-excision interactions, only plants that flowered were used since excision could only influence flowering plants. This necessarily reduces power to look at insecticide effects, so where insecticide effects were not found, I tested for insecticide main effects alone using all the data. Model selection was identical to that described in the previous experiment, and of the 23 original models, final models are reported in Table 2 when treatment effects are significant; non-significant models are noted in the text.

Correlations between size and flowering traits

Analysis

Control plants, pooled across both experiments to increase power, were used to examine correlations between size and flowering traits. Control plants received no tissue removal in the first experiment and received water spray with no stigma excision in the second experiment. Pearson correlation coefficients (r) were calculated among plant height, stem number and flower number. Stem and flower number were log transformed to meet assumptions of normality of residuals.

RESULTS

Herbivore and pollinator response to plant size and flowering phenotype

In the treatment year 50% of plants flowered, with those that did producing 24.5 ± 6.1 flowers per plant. Average percent damage was 7.5 ± 0.4 per plant. Both removal treatments affected fruit number in the treatment year (Table 1, Figure 2). Contrasts show that stem removal increased fruit number on remaining stems relative to control plants ($z = -3.007$, $P = 0.003$), while flower removal decreased fruit number relative to non-flower removal (control plus stem removal) plants (Figure 2A, $z = -2.025$, $P = 0.043$). There were no effects of stem or flower removal on damage by insect herbivores (Figure 2C) or mammalian herbivores (Figure 2B), although there was a trend for both removal treatments to reduce mammalian browsing. In general, deer browsing is not trivial in this habitat: in our study, 35% of plants had at least one stem browsed in the treatment year, and 74% were browsed in the following year. There was minimal plant loss in either year, due to trampling by deer, bears, or cattle, and probably reflected only above-ground biomass loss rather than plant mortality.

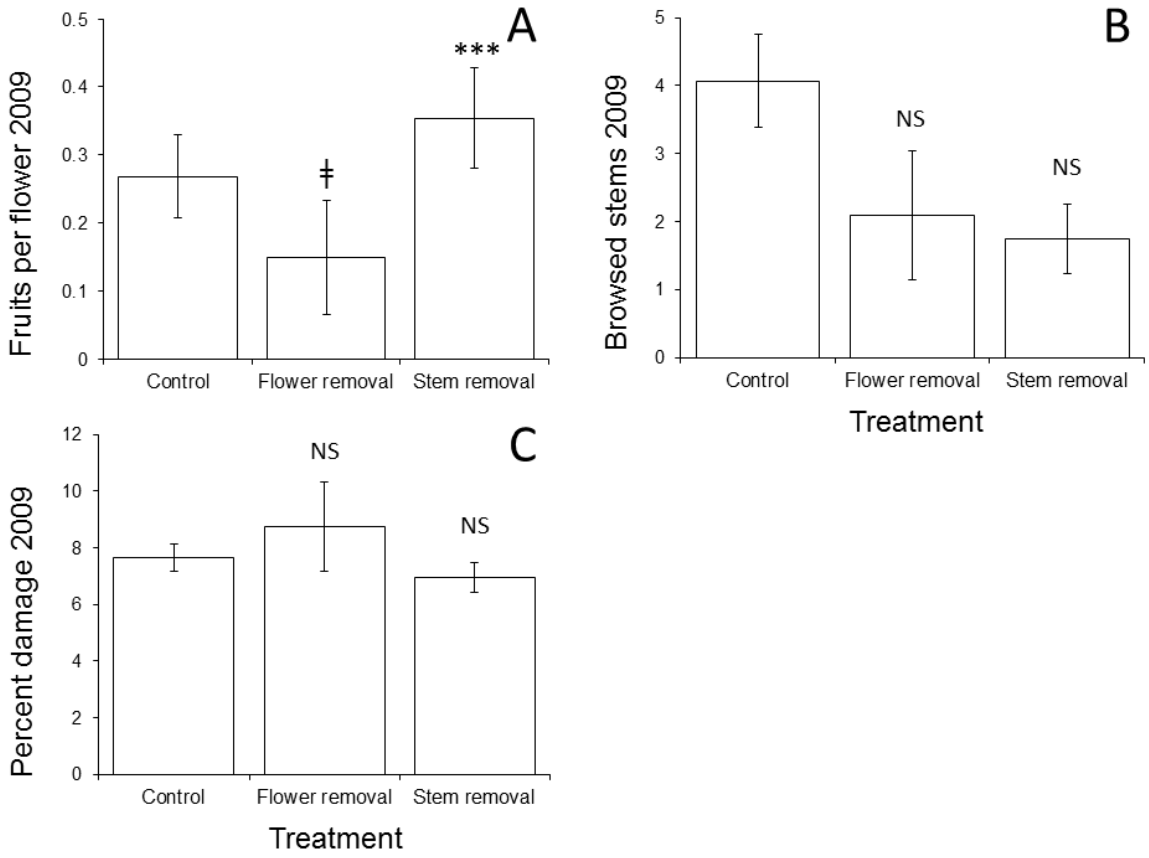

Figure 2. Effects of stem and flower removal on plant traits. (A) Stem removal increases fruit number in 2009 relative to control ($P = 0.003$). Flower removal decreases fruit number relative to all other plants ($P = 0.043$). Figure shows proportion fruits/flower since model accounts for flower number. (B) Flower and stem removal non-significantly decrease browsed stems in 2009. (C) Removal has no effect on percent leaf damage in 2009. Error bars show ± 1 SE; *** indicates $P < 0.0001$; ‡ indicates significance when compared control plus plants in the other treatment group.

Effect of herbivore damage and pollination on flowering and size traits

In the treatment year (2009), 44% of plants flowered, with those that did producing 38 ±7.8 flowers per plant. Across all treatments, plants were an average of 73 ± 1.4 cm tall and produced 22 ± 1.2 stems per plant. In the following year (2010), 51% of plants flowered, with those that did producing 93.5 ± 14.5 flowers per plant. Across all treatments, plants were an average of 72.4 ± 1.7 cm tall and produced 18.2 ± 1 stems per plant. There was minimal plant loss in either year, due to trampling by deer, bears, or cattle, and probably reflected only above-ground biomass loss rather than plant mortality.

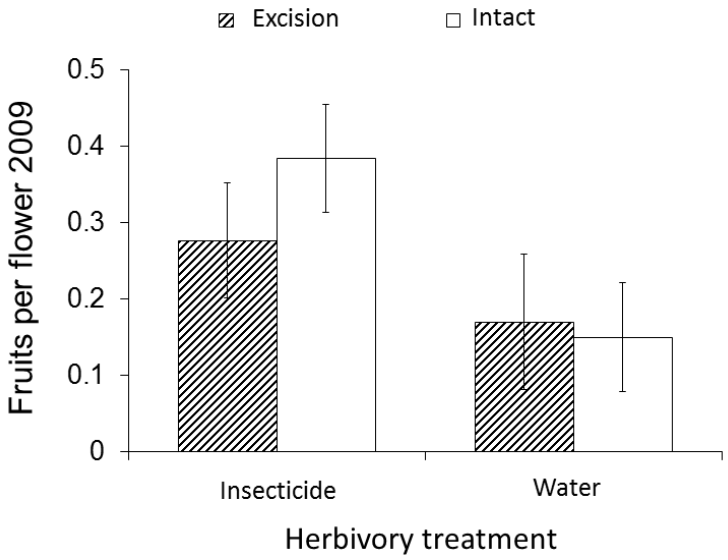

Figure 3. A damage-by-pollination interaction on fruit number occurred within years: stigma excision was more effective in insecticide sprayed plants ($P = 0.014$). Figure shows proportion fruits per flower (error bars show ± 1 SE) since flower number is accounted for in model.

Insecticide effectively decreased herbivory in the treatment year, reducing cumulative percent damage per plant by 57% (t = -7.44, $P < 0.001$). There were no residual effects of insecticide on damage received in the following year when plants were not sprayed. Although the final insecticide application occurred on 13 July 2009, cumulative damage levels on insecticide treated plants remained substantially reduced relative to control plants for the remainder of the season (Figure 1). Stigma excision (which was received by most but not all flowers on stigma-removal plants, so that fruit production was reduced but not eliminated) reduced fruit number in the treatment year only in plants also receiving insecticide (interaction term $P = 0.014$), while plants exposed to natural herbivory produced very few fruits regardless of excision treatment (Figure 3).

Using all the data to examine main effects of insecticide relative to control plants, insecticide increased flower number (t = 4.02, $P = 0.008$), number of flowering stems (z = 2.58, $P = 0.01$; Figure 4A and 4B), and fruit number (z = 4.24, $P < 0.0001$, Figure 4C). Insecticide decreased stem number in the treatment year (t = -2.67, $P < 0.0001$, Figure 4D) and the following year (t = -2.61, $P = 0.01$, Figure 4E) and height in the following year (t = -2.53, $P = 0.013$, Figure 5F).

Stigma excision increased percent leaf damage in the treatment year by 40% relative to plants with intact stigmas ($F_{1, 52} = 3.8$, $P = 0.056$, Figure 5A). In the year following excision, excision increased height by 11% ($F_{1, 53} = 4.06$, $P = 0.049$, Figure 5B), increased stem number by 39% ($F_{1, 52} = 4.12$, $P = 0.048$, Figure 5C), and decreased browsed stems by 42% ($\chi^2 = 5.44$, $P = 0.02$, Figure 5D).

Correlations between size and flowering traits

Flowering and size traits were generally positively correlated. For unmanipulated (control) plants pooled over both experiments, stem number and height were positively correlated in both years of the study (2009 r = 0.28, $P = 0.007$; 2010 r = 0.23, $P = 0.035$). Flower

number correlated positively with height (r = 0.61, $P < 0.0001$) and stem number (r = 0.396, $P = 0.001$) in the second year. All three measures were positively correlated across years (height: r = 0.71, $P < 0.0001$; stem number: r = 0.81, $P < 0.0001$; flower number (for plants that flowered in both years) r = 0.61, $P = 0.037$).

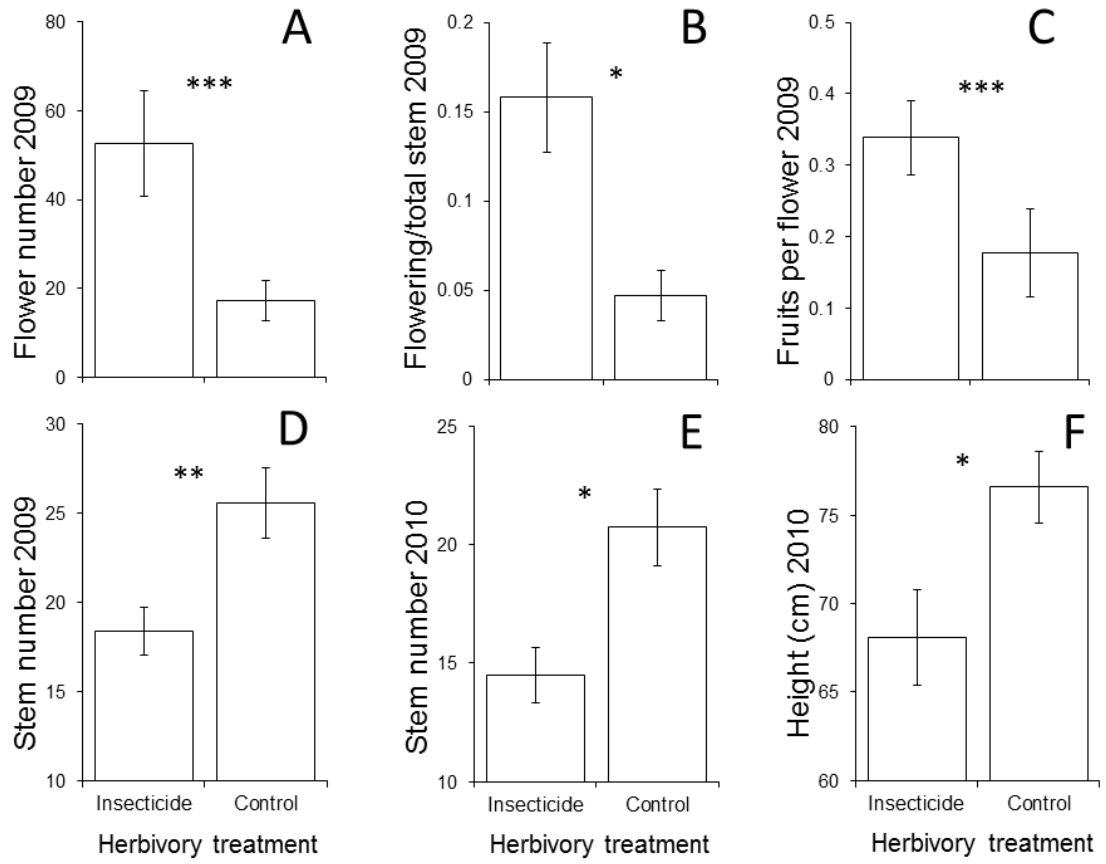

Figure 4. Effects of insecticide on plant traits. Insecticide (A) increases flower number threefold ($P < 0.0001$), (B) proportion of flowering stems by 140% ($P = 0.011$), and (C) proportion of fruit per flower ($P < 0.0001$) within years, relative to control plants. Insecticide decreases stem number (D) within ($P = 0.008$) and (E) across ($P = 0.013$) years, and (F) decreases height across years ($P = 0.012$), relative to control plants. Figures show proportion flowering stems and fruits because stem number and flower number, respectively, are accounted for in the models. Error bars show ± 1 SE. * indicates $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 1. Effects of stem and flower removal treatments on plant and forager responses. Fruit number responses used flowering plants only. Models that explained significant variation in data are reported.

Final model	Response	df	χ2	*P*
Fruit number 2009 = Removal treatment * Flower number 2009	Removal treatment	2	10.464	**0.005**
	Flower number 2009	1	20.678	**<0.001**
	Treatment*Flower number 2009	2	9.172	**0.01**
	Residual	42		

Table 2. Effects of insecticide and excision treatments on plant and forager responses. Degrees of freedom reflect dataset used; df = 119 or 122 used all plants, df = 48, 49, 52, or 53 used flowering plants only. Models that explained significant variation in data are reported.

Final model	Response	df	F or χ2	*P*
Browsed stems 2010 = Excision * Stem number 2010	Excision	1	5.439	**0.02**
	Stem number 2010	1	22.329	**<0.001**
	Excision*Stem number 2010	1	3.271	0.071
	Residual	49		
Flowering stems 2009 = Insecticide + Stem number 2009	Insecticide	1	6.616	**0.01**
	Stem number 2009	1	1.090	0.3
	Residual	122		
Fruit number 2009 = Insecticide + Excision + Flower number 2009, plus 2-way interactions	Insecticide	1	30.003	**<0.001**
	Excision	1	0.928	0.335
	Flower number 2009	1	71.448	**<0.001**
	Insecticide*Excision	1	9.667	**0.002**
	Insecticide*Flower number 2009	1	36.857	**<0.001**
	Excision*Flower number 2009	1	1.564	0.211
	Residual	48		
Flowering stems 2010 = Insecticide * Stem number 2010	Insecticide	1	3.627	0.057•
	Stem number 2010	1	30.205	**<0.001**
	Insecticide*Stem number 2010	1	5.7139	**0.017**
	Residual	49		
Stem number 2009 = Insecticide	Insecticide	1	7.2266	**0.008**
	Residual	122		
Percent damage 2009 = Insecticide + Excision	Insecticide	1	15.567	**<0.001**
	Excision	1	3.8354	0.056•
	Residual	52		
Flower number 2009 = Insecticide + Excision	Insecticide	1	16.1618	**<0.001**
	Excision	1	0.7734	0.383
	Residual	52		
Height 2010 = Excision	Excision	1	4.0649	**0.049**
	Residual	53		
Height 2010 = Insecticide	Insecticide	1	6.3663	**0.013**
	Residual	122		
Stem number 2010 = Insecticide * Excision	Insecticide	1	6.5653	**0.013**
	Excision	1	4.1162	**0.048**
	Residual	52		
Stem number 2010 = Insecticide	Insecticide	1	6.7866	**0.01**
	Residual	119		

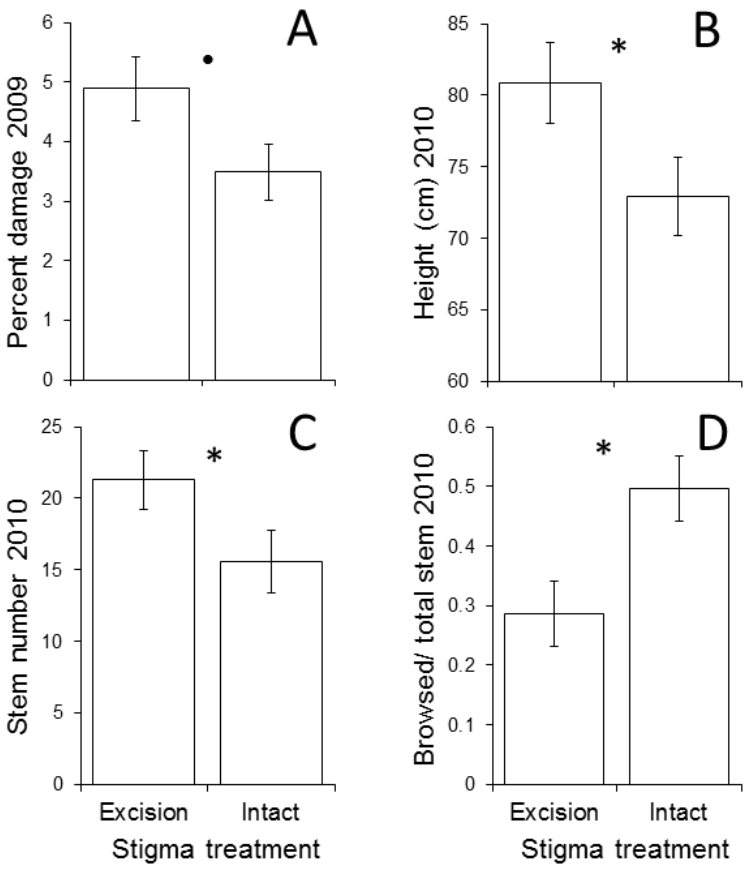

Figure 5. Effects of stigma excision on plant traits Stigma excision increased (A) percent leaf damage in 2009 relative to stigma intact plants ($P = 0.056$); (B) height in 2010 relative to stigma intact plants ($P = 0.049$); (C) stem number in 2010 relative to stigma intact plants ($P = 0.048$); and (D) decreased browsed stems in 2010 relative to stigma intact plants ($P = 0.02$). Data are from flowering plants only. Error bars show ± 1 SE.

DISCUSSION

Our results reveal considerable, but not necessarily balanced, feedback between foragers and plant traits both within and between years. Reduction in pollen receipt and insect damage (through stigma excision and insecticide) both affected plant traits. However, while fruit set responses suggest that pollinators were responsive to both insect damage (manipulated by insecticide) and plant size and flowering traits (manipulated by stem and flower removal), insect herbivores responded only to pollen receipt (stigma excision) and were unexpectedly unresponsive to other plant traits.

Insect damage, as manipulated by insecticide, had an important effect on flowering traits and pollination, decreasing flower number, flowering stems, and fruit number within years (Figure 3A-C). The decrease in fruit number with damage suggests that damage reduced pollinator visits or pollen receipt per flower. Because the model for fruit number accounts for flower number as a covariate (Table 2), decreases in fruit number were not due simply to damage decreasing the number of flowers. Also, because damage increased stem number, the decrease in

fruit number is likely pollinator-driven, rather than resource-driven. Results from flower removal treatments also suggest that pollinators prefer plants with more flowers (Figure 2A). Indirect effects of herbivores on pollinators are not surprising. Leaf herbivores have been shown to decrease pollinator visitation within years by reducing flower size and number (Lehtilä and Strauss, 1997; Steets and Ashman, 2004). It is also possible that leaf herbivores decreased fruit number by reducing resources available for maturing seeds (e.g., Mothershead and Marquis, 2000), although positive effects of herbivores on plant size in the treatment year (Figure 4D), possibly due to reallocation of resources from the rhizome to tolerate herbivory, suggest that resource limitation by herbivores was not severe.

In this system, indirect effects may be reciprocal. Reduced pollen receipt (due to stigma excision) increased damage by insect herbivores in the treatment year (Figure 5A). Although effects of pollen receipt on damage have been demonstrated for mammalian frugivores (Herrera, 2000), to our knowledge they have not been demonstrated for leaf herbivores. Pollination may result in resources being diverted from defense to fruit maturation, although the presence of induced defenses in this species has not been tested. An alternative explanation is that the damage associated with stigma excision itself caused a change in plant defense or nutrient quality. While mechanical damage is often used experimentally, there is evidence that mechanical damage does not have a strong effect on defenses (Massey *et al.*, 2007), and the tiny amount of tissue removed in these experiments may not reach the damage threshold required to induce defenses in some plants (e.g., Underwood, 2000; Massey *et al.*, 2007). If pollination were diverting resources from plant defense, I would expect higher damage in intact stigma treatments, which I did not find. A possible mechanism for increased damage following pollination is increased attractiveness of fruiting plants to herbivores, although this experiment is not capable of testing that possibility. The fact that I found negative effects of pollen receipt on leaf damage even though our power to detect effects of stigma excision may have been reduced due to low flower number suggests that indirect effects of pollinators on leaf herbivores could be important and should be considered in other systems.

Although the insecticide-by-excision interaction (Figure 3) may indicate that damage is an important factor for fruit production (see also Figure 4C), pollen receipt is still likely the primary driver for sexual reproduction, and therefore fitness, in *C. angustifolium*. The continuing effects of stigma excision in the year following treatment (Figure 5B-C) also indicate that pollen receipt had important influences on resource allocation. Most effects of stigma excision manifested the year after the treatment was imposed, but this was not unexpected because the timing of pollination and subsequent fruit set at the end of the growing season make strong effects on resource allocation more likely in the next year. It is somewhat surprising, however, that across year pollen receipt influences plant size traits rather than flowering traits. Plants that received less pollen due to stigma excision had more stems and achieved taller stature across years (Figure 5B-C), suggesting that lack of pollen receipt and subsequent allocation to fruit formation may allow, or possibly prompt, greater investment in size the next year.

The across-year effects reported here (plant size response to herbivores, Figure 4D-F; plant size response to pollen receipt, Figure 5B-C) likely represent changes in resource investment to new stems produced each year from stored resources. There was no direct carry-over of either insecticide or excision treatments, i.e., insecticide treatments did not affect leaf damage in the following year, and plants in the stigma excision treatment produced normal stigmas in the following year (data not shown).

Across-year effects of herbivores are not unknown. For example, leaf herbivory increased stem number but decrease reproduction across years in *Melaleuca quinquenervia (Pratt et al., 2005)*, and after an initial negative effect, increased both stem growth and flower production across years in *Cornus florida* (Sacchi and Connor, 1999). Across-year effects of fruit production include a subsequent-year decrease in fruit production in the rewarding orchid *Gymnadenia conopsea* (Sletvold and Ågren, 2011) and a subsequent-year decrease in plant size in the orchid *Tipularia discolor* (Snow and Whigham, 1989). In our study, across year responses to herbivory paralleled within-year responses (Figure 4D-E), but most responses to stigma excision only manifested across years (Figure 5). The fact that both foraging types had across year effects in our study suggests that multiple-year studies will be necessary to determine forager effects in iteroparous perennial species. Single-year studies might misinterpret the ultimate cause of variation in reproductive effort or underestimate the effect of damage or pollination. My two-year study of plant responses to herbivory and pollination are a good first step toward understanding of the longer term effects of foragers on plant traits. However, this length of study does not capture lifetime responses in long-lived perennials such as *C. angustifolium.* Understanding longer term effects likely requires either lifetime studies of long-lived plants or sufficient across-year responses to build a matrix model to project responses through the plant life cycle.

I found that herbivore damage increased height and stem number (Figures 4D-F) both in the year of damage and in the following year, indicating a compensatory response to damage. Compensatory responses to damage have been found in other studies, as some plants can produce more branches (Paige and Whitham, 1987) or replace inflorescences (Pilson and Decker, 2002) in response to apical damage. Compensation in response to leaf damage is less common, and those studies reporting compensation typically address only within-year effects (but see Brody *et al.*, 2007 for life table predictions of damage effects across lifespan in Ipomopsis aggregata). The long-term consequences of such compensation or overcompensation are unclear. To our knowledge there is no evidence of overcompensation persisting for the lifetime of a long-lived iteroparous plant like *C. angustifolium*. Overcompensation across two years does not establish a lifetime pattern, but it does indicate the potential for long-term effects. While size overcompensation in response to herbivory is often seen as a mechanism to tolerate or take advantage of damage, in this system there may be little advantage to being larger, since stem removal resulted in no change in insect damage, reduced (although not significantly) deer browsing, and an unexpected increase in within year fruit number (Figure 2A-C). These results seem to indicate that larger plants are more noticeable or attractive to deer herbivores, but not to insect pollinators or herbivores.

Although herbivores positively affect size and negatively affect flowering within years (Figures 3A, B, and D), it seems unlikely that this occurred through allocation tradeoffs between size and reproductive traits. Positive correlations between size and flowering traits within years, and between traits across years, suggest no trade-offs between size and flowering in this system. This finding is in agreement with previous studies where size and flowering traits were found to correlate positively (e.g., Herrera, 2004).

The unexpected response of fruit set to size manipulation brings up a possible confounding effect of stem and flower removal treatments. While manipulations were intended

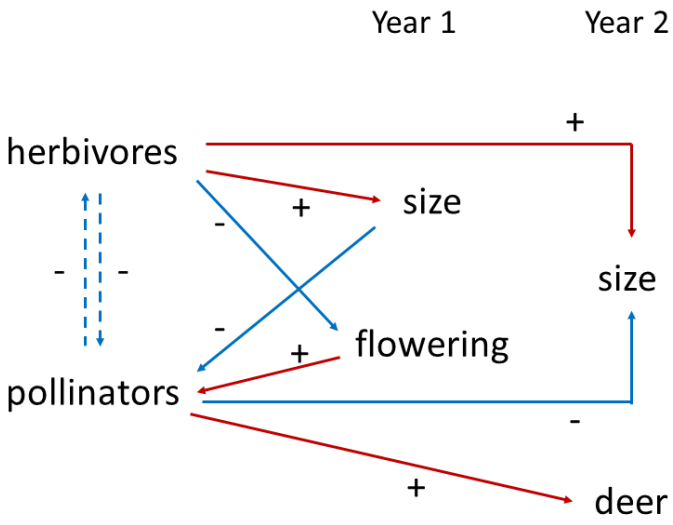

Figure 6. Within and across year plant-forager relationships. Red arrows indicate a positive effect, blue arrows a negative effect. Dashed arrows show an indirect effect.

to alter plant appearance to foragers, removal of biomass may incur secondary plant responses, including reallocation of resources to other functions or induced defenses. Clipping distal portions of plant stems to mimic deer browsing can initiate reproductive compensation (Paige and Whitham, 1987). In this experiment, while the change was not significant, plants in stem removal treatments did show a 26% increase in proportion flowering stems per plant within years relative to non-stem removal plants, and this may have contributed to increased pollinator attraction and increased fruiting relative to non-stem removal plants. While damage can also induce plant defenses, mechanical tissue removal may fail to activate defense pathways (Walling, 2000), and the lack of insect herbivore response to stem clipping suggests that stem removal did not induce plant defenses in this experiment.

CONCLUSIONS

My results suggest that the overall effects of herbivores and pollinators on plant size and flowering traits contribute to intermediate trait expression. The general effect of herbivores in this study was to decrease flowering traits (Figure 4A-C) and increase size traits (Figure 4D-F), so that herbivory should result in larger plants with fewer flowers (Figure 6). Whether these effects would manifest in long-term selection pressures on plants is unclear from these experiments, given the lack of herbivore response to manipulated plant phenotype. Pollinators have a negative influence on plant size (Figure 5B and 5C, Figure 6), so pollination should result in expression of smaller plants. However, because pollinators appear to respond negatively to plant size, the strength of this effect should diminish as plant size decreases. Because pollinators respond positively to flower number and negatively to plant size (Figure 2A) and have positive effects on plant fitness, pollinators might impose selection on plants to produce more flowers or

smaller stature. Since herbivory results in large plants with few flowers, the joint effects of pollinators and herbivores in this system may contribute to intermediate values of plant size and flowering traits. Studies quantifying plant fitness and plant-forager interaction strengths, ideally across plant lifetimes, are necessary to determine the net outcome of herbivore and pollinator effects on plant traits.

CHAPTER THREE

FORAGER VARIABILITY AND THE EVOLUTION OF PLANT ALLOCATION SCHEDULE

INTRODUCTION

Abiotic and biotic factors that affect plant fitness are often highly variable in time. This includes herbivory (Huntly, 1991), which is often detrimental to plant fitness (Marquis, 1984; Agrawal, 1998), and pollination (Horvitz and Schemske, 1990; Price *et al.*, 2005), which is beneficial to plant fitness in outcrossing species. Herbivory and pollination can vary temporally within years, so that plants might experience higher levels of herbivory or pollination during some time periods than others (e.g., Ashman and Stanton, 1991; Filip *et al.*, 1995). Herbivory and pollination can also vary among years, so that herbivory and pollination might peak at different times of the season from year to year (e.g., Root, 1996). Plants should be under selection to evolve patterns of trait expression that will maximize fitness under variable herbivory and pollination.

To deal with variability in herbivory and pollination, plants may evolve an optimal allocation schedule. Here, I define "allocation schedule" as the time of the season when a plant begins allocating resources to flowering *and* the time of the season when a plant stops allocating resources to growth. For example, plants can start allocating resources to reproduction either early in the season or late in the season (the first parameter of the allocation schedule) and can stop allocating resources to growth either immediately after allocation to flowering begins or on some later day (the second parameter of the allocation schedule). A plant that stops allocating resources to growth immediately after beginning allocation to flowering is expressing a "bang-bang" schedule (Cohen, 1971) where the allocation switch is abrupt, while a plant that continues allocating resources to growth after flowering begins is expressing a gradual or "graded" (King and Roughgarden, 1982) schedule. Either the bang-bang or gradual schedule can occur early or late in the season, although the later the switch to reproductive allocation, the less gradual the schedule can be.

There is a substantial body of theory predicting how environmental variability and predictability should influence the evolution of plant traits. Under no variability we would expect a single phenotype adapted for the constant environment. For the timing of switching allocation to reproduction in plants, the bang-bang allocation schedule is predicted to be favored when the environment does not vary (Cohen, 1971). If the environment is variable across years but accompanied by reliable cues as to what the environment will be, theory suggests that plants should evolve adaptive plasticity to express the optimum phenotype in each environment (Via and Lande, 1985; Moran, 1992). In the absence of reliable cues, i.e. when the environment varies stochastically, we would expect a bet-hedging strategy that maximizes fitness by reducing variance in fitness across all environments according to their probability of occurrence (Hopper, 1999; Wong and Ackerly, 2005). Wong and Ackerly (2005) modeled annual plants with variable predictability in end-of-season to compare plastic and bet-hedging strategies. They found that with a perfect cue a bang-bang schedule is favored, and as predictability decreases optimal schedules become more gradual.

Models incorporating stochastic (unpredictable) variability in end-of-season show the evolution of a gradual switch or multiple switches between growth and reproduction in annual

plants (Cohen, 1971; King and Roughgarden, 1982; Satake *et al.*, 2001). In natural populations of some desert annuals, for which end-of-season is unpredictable, flowering begins early in the season and coincides with growth (Cohen, 1971; King and Roughgarden, 1982). Variability in end-of-season could be caused by a range of factors, including sudden and lethal herbivory such as might occur by mammalian grazing. In many cases, however, particularly with insect herbivores, herbivory is chronic and sub-lethal. Chronic sub-lethal herbivory still allows a plant to grow and reproduce after damage, while sudden lethal herbivory does not, so it might be expected that plants will evolve differently under sub-lethal herbivory. Despite this, there is little theory predicting evolution under chronic sub-lethal of herbivory. Yamamura *et al.* (Yamamura *et al.*, 2007) modeled annual plants under chronic sub-lethal grazing, but focus on magnitude rather than variability in grazing. They found that increasing vegetative grazing delays flowering longer when growth is a non-linear function of plant size. Models of perennial plants in unpredictably variable environments are less common than models of annuals, but those that have been done predict that plants will evolve multiple switches between growth and reproduction or gradual schedules of allocation when the end-of season is variable and unpredictable (Perrin and Sibly, 1993).

I used a simulation model to examine the evolution of allocation schedule in annual and perennial plants in stochastically variable herbivory and pollination environments, that is, evolution in a bet-hedging scenario. The perennial plant is a variation of the annual plant where resource allocation switches to both reproduction and storage, and storage carries over to increase initial plant size at the start of the next season. This allows me to determine if perennial plants are likely to evolve a different allocation schedule because they can be influenced by the previous years' environment. I first explored evolution of allocation schedule in perfectly predictable environments, that is, herbivory and pollination both vary within a season but peak at same time each season. In this case, based on previous theory, I expect plants to evolve a single optimal allocation schedule that maximizes herbivore tolerance and total flower production across the season. I then explored evolution of allocation schedule in environments of increasing stochastic variability, that is, herbivory and pollination both peak at an unpredictable time each season. If plants evolve similarly in response to variability in my model and to variability in unpredictable lethal disturbances, then I expect that both annual and perennial plants will evolve gradual allocation schedules.

While the evolution of allocation schedule for annuals, and occasionally perennials, in variable environments has been considered previously (King and Roughgarden, 1982; Perrin and Sibly, 1993; Satake *et al.*, 2001; Wong and Ackerly, 2005; Yamamura *et al.*, 2007), two differences set my model apart. First, variability in a disturbance is generally incorporated as an acute and severe disturbance (e.g., flooding, Satake *et al.*, 2001; or end-of-season, Wong and Ackerly, 2005; but see Yamamura *et al.*, 2007 for chronic grazing pressure). While some types of herbivory do involve an acute and severe loss of biomass, insect herbivory is often a continuous condition resulting in sub-lethal size reduction. Herbivory as a chronic condition is rarely incorporated into models of plant evolution, but is likely to select for different allocation schedules because growth and reproduction can still continue, although at reduced levels, after herbivory.

Second, variability in a disturbance is generally incorporated into models independently of other conditions that benefit plant fitness. In this model, I examine the joint effects of variability in antagonists (herbivory) and mutualists (pollination) on plant fitness. Host plants interact with both antagonists and mutualists, which together can have important fitness

consequences (Lehtilä and Strauss, 1997; Steets and Ashman, 2004; Strauss and Murch, 2004). I intentionally correlate variability in herbivory and pollination, that is, both are either highly variable or less variable but do not necessarily peak at the same time, in order to address the joint effects of plant antagonists and mutualists on evolution of allocation schedule. Although factors influencing herbivore and pollinator abundances are complex, both herbivores and pollinators can be similarly affected by abiotic conditions like temperature and humidity (Gullan and Cranston, 2000). The consequences of varying herbivory and pollination together are too complex to predict, but my model will be a first step toward predicting the effects of simultaneous increases in the variability of both a positive and negative influence on plant fitness.

METHODS

To examine selection on allocation schedule in annual and perennial plants in stochastically variable herbivory and pollination environments, I developed a simulation model. Using this model, I looked at annual and perennial plants separately, and ran the model at four levels of environmental variability, from no variability to very high variability, for a total of eight different simulation types. Each run of the model gives the geometric mean of annual fitness for every a range of allocation schedules (see "Defining allocation schedule" below) to growth and reproduction (or reproduction and storage, in the perennial plant).

Defining plant functions

The model follows individual plants that grow, flower, and experience herbivory and pollination within a single growing season (a year) consisting of 100 time steps (days) (Figure 7, see Table 3 for a list of model parameters and values). The following equation describes an individual's size at each time step (denoted by subscript *i*)

Eq. 1 $$S_{i+1} = S_i + (S_i * G_i * g_i) - (c_f * F_i) - (c_{fr} * W_i) - (c_s * S_i) - H_i$$

where the next day's size (S_{i+1}) is a function of current size (S_i), a growth rate (G_i), the fraction of resources allocated to growth (g_i), a cost of producing flowers given by a constant fraction (c_f) of current flower number (F_i), a cost of producing fruit given by a constant fraction (c_{fr}) of current fruit number (W_i), a cost of growth given by a constant fraction (c_s) of current size (S_i), and the herbivory function (H_i). Plants also lose some portion of size to make flowers and fruits, and to maintain growth. This model assumes that herbivores reduce plant size but do not induce defenses (there is no allocation to defense).

Growth is logistic so that smaller plants have a higher per-mass growth rate than large plants and growth rate declines in very large plants, which will eventually reach a maximum size.

Eq. 2 $$G_i = [G_{min} * (1 - (S_i / S_{max}))^{-1/2}] * g_i$$

There is a minimum growth rate (G_{min}) and a maximum size (S_{max}), and growth rate is adjusted to account for proportion of resources allocated to growth (g_i). Growth rate is bounded by 0 and 1. Each day an individual plant may also produce flowers. The presence of flowers is determined by an allocation function (see "Defining allocation schedule" below). The number of open flowers is given by

Eq. 3 $F_i = F_{i-1} + (F_{new} * S_i * f_i) - (F_{i-1} * 1 / t_{open})$

where number of open flowers (F_i) is based on flowers remaining from the previous day (F_{i-1}), new flowers (F_{new}), plant size, the fraction of resources allocated to flowering (f_i), and the time that flowers remain open (t_{open}). F_{new} is a binary variable (0 or 1) that will allow flower production only if resources are currently being allocated to flowers (see "Defining allocation schedule" below). The decay rate of flowers ($F_{i-1} * 1 / t_{open}$) controls how long flowers stay open; a large t_{open} means that flowers are open for many days, and therefore a plant will have many open flowers relative to a plant with a small t_{open}.

Perennial individuals have a storage function that annuals do not, given by

Eq. 4 $R_i = R_{i-1} + R_{new} * c_r * S_i * r_i$

where the amount in storage on a given day (R_i) is determined by previous storage size (R_{i-1}) plus new storage (R_{new}), a constant fraction (c_r) of current size, and the fraction of resources allocated to storage (r_i). As in the flower function (Eq. 3), R_{new} is a binary variable (0 or 1) that will allow storage production only if resources are currently being allocated to storage (see "Defining allocation schedule" below). This function describes a situation in which larger plants acquire more resources and move a fraction of those resources to a storage organ which saves those resources for the remainder of the season.

Daily fruit production is given by

Eq. 5 $W_i = F_i * P_i$

where fruits (W_i) are given by the number of open flowers and the pollination function (P_i). Average annual fruit set (over 100 single-year iterations for the annual plant or averaged over 100 consecutive years for the perennial plant) represents plant fitness. Based on preliminary results using 25, 50, 75, and 100 years, I determined that a 100-year lifetime is sufficient for consistent results.

Daily herbivory (H_i) and pollination (P_i) are modeled as Gaussian distributions (e.g. Figure 10A), with the distribution mean as the day of peak herbivory or pollination. With no variability in timing of herbivory and pollination, herbivory peaks at day 30 and pollination peaks at day 60 of the season. These peak days are based on the assumption that plants can't be pollinated until they make flowers but can be eaten from the beginning of the growing season.

Annual plants start each year with initial size = 10 and have no storage capability. In contrast, perennial plants start the first year of their lifetime with initial size = 10 and allocate some resources to storage. A fraction of stored resources is carried across years so that perennial plants have larger initial size at the beginning of the subsequent growing season. Initial size in the subsequent year is the final storage size of the previous year ($S_1 = R_{100}$). For simplicity I include no cost of respiration during dormancy, but there is a cost associated with allocating resources to storage (some resources are lost in the process). Herbivory reduces plant size but is not severe enough to cause plant death. Otherwise, mortality is not explicitly incorporated into my model.

Table 3. Model parameters and values

G_i	daily growth rate
G_{min}	minimum growth rate = 0.1
S_1	initial size = 10
S_i	daily plant size
S_{max}	maximum plant size = 5000
F_i	daily flower number
F_{new}	presence of new flowers (0 or 1)
t_{open}	duration of open flowers = 3 days
R_i	new storage produced each day
R_{new}	presence of new storage units (0 or 1)
W_i	number of fruits produced each day
g_i	proportion of resources allocated to growth
f_i	proportion of resources allocated to flowering
r_i	proportion of resources allocated to storage
c_s	proportion of size representing maintenance cost = 0.05
c_f	proportion of flowers representing cost of flowers = 0.2
c_{fr}	proportion of fruits representing cost of fruits = 0.5
H_i	daily herbivory
P_i	daily pollination
T_s	day of the season that allocation to reproduction and storage begins
T_e	day of the season that allocation to growth ends

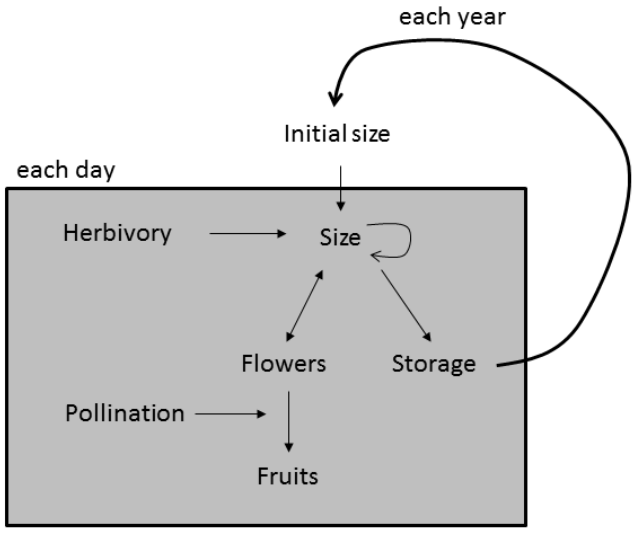

Figure 7. Diagram of simulation model. A plant undergoes daily functions of growth, reproduction, and (in the perennial plant), storage, and experiences daily herbivory and pollination. In the perennial plant, storage is converted to initial size at the beginning of the next year.

Defining allocation schedule

The model runs a range of combinations of allocation to growth and reproduction. For the annual plant, the allocation schedule describes the transition from growth to reproduction. For the perennial the schedule describes the transition from growth to both reproduction and storage. The proportion of resources allocated to growth (g_i, Figure 8) is described by two parameters: the day of the season that allocation to reproduction and storage begins (T_s) and the day of the season that allocation to growth ends (T_e). Allocation to growth is 1 before the reproductive or reproductive/storage period begins ($i < T_s$) and 0 after the growth period ends ($i > T_e$). Between T_s and T_e, allocation to growth is a decreasing straight line (Figure 8).

At the start of a season individuals allocate all resources to growth, and initiate allocation to other functions at some later point in the season. The functions other than growth receive some ratio of all the remaining resources not used for growth. The transition from growth to other functions can occur at any time after the first day, and can be abrupt or gradual. I calculated the fitness (geometric mean of annual fruit production) for all possible allocation schedules to assess fitness consequences (i.e., T_s ranged from 1 to 99 and T_e from 2 to 100 with $T_s < T_e$).

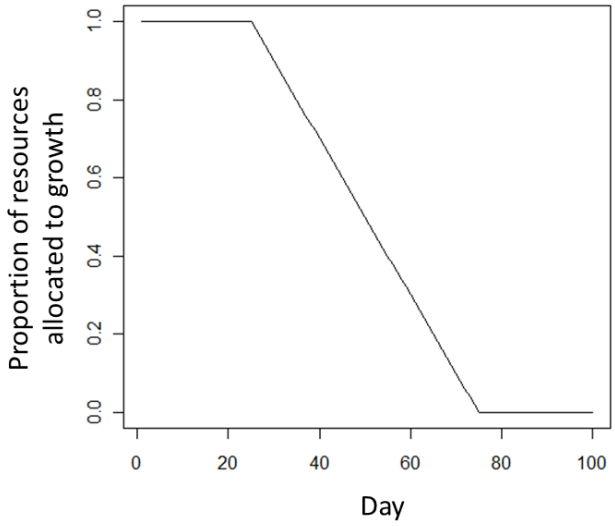

Figure 8. Sample allocation schedule. This is a gradual allocation schedule where allocation to reproduction begins at day 25 and allocation to growth ends at day 75.

For perennial plants, all models were run with a constant 1:3 ratio of allocation to flowering and storage. That is, of the resources not allocated to growth, 25% were allocated to flowering and 75% were allocated to storage. This ratio reflects the fact that in real plants storage organs generally have greater biomass than flowering parts.

To compare fitness across allocation schedules, I produce a fitness surface (Figure 9) that shows average annual fitness for every possible allocation schedule (i.e., every possible combination of T_s and T_e). Because allocation to growth must end sometime after allocation to reproduction begins, there will only be fitness values for the upper left half of the fitness surface (other values are impossible). The surface gives the optimal allocation schedule (the peak of the surface) and the strength of selection on that schedule (the slope of the surface approaching that

peak). Points near the diagonal represent bang-bang allocation schedules, and those further from the diagonal represent increasingly gradual allocation schedules. To visualize the slope, I identify the fitness values at least 90% of the maximum in red (Figure 9). A broader area of red represents a flatter surface with weaker selection for the maximum, while a smaller area of red represents a more steeply sloped with stronger selection for the maximum.

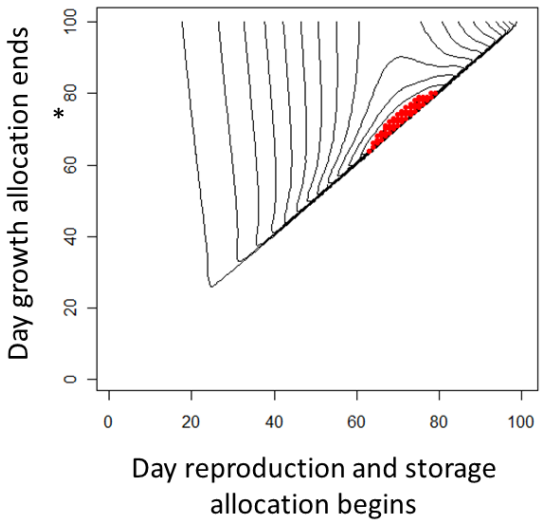

Figure 9. Sample fitness surface. Fitness surface representing mean annual fruit set of a plant for a range of possible allocation schedules. Contours connect points with identical fitness, which increase to some peak. All allocation patterns with fitness values at least 90% of the maximum value are colored red. The * marks the fitness maximum.

Variability in herbivory and pollination

Under perfectly predicable variability, herbivory and pollination vary from day to day within a season, but peak at same time each season (Figure 10A). Under stochastically variable herbivory and pollination, the model selects a value for time of peak insect abundance from a uniform distribution with either a narrow range of values (for low across-year variability) or a wide range of values (for high across-year variability) with equal means (Figure 10B, Table 4).

Table 4. Parameters defining variability in simulations. Parameters are taken from a uniform distribution with the given variances. Values differ for herbivory and pollination at intermediate and high levels because the mean of the distribution are offset (at 30 and 60, respectively), so the range of herbivory is slightly more constrained than the range of pollination.

Range of variability	**Model parameter**
Low	$\sigma^2 = 8.33$
Intermediate	$\sigma^2 = 208.33$ (herbivory), 352.08 (pollination)
High	$\sigma^2 = 408.33$ (herbivory), 602.08 (pollination)

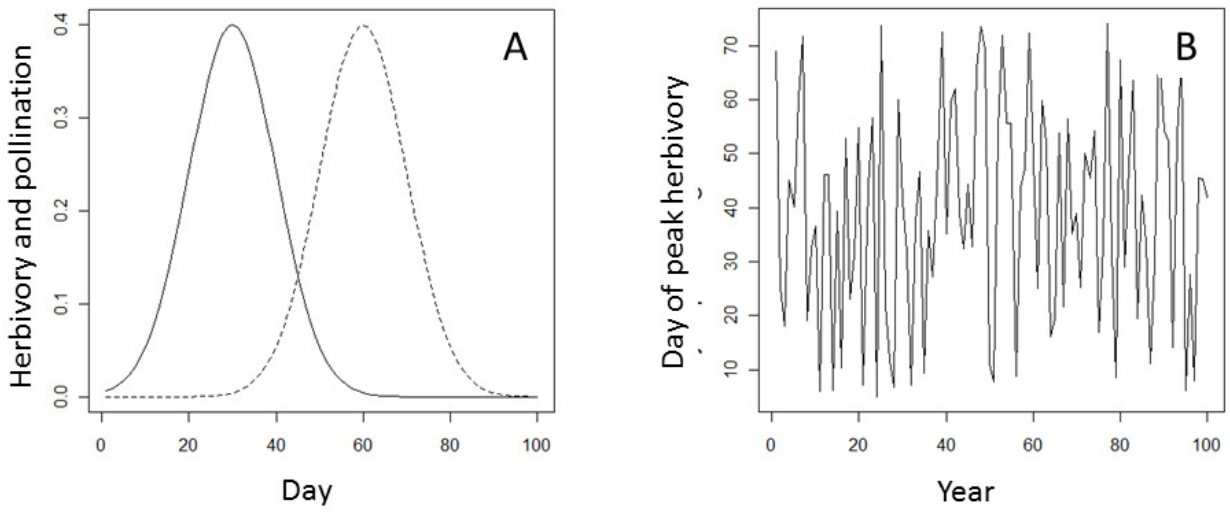

Figure 10. Herbivory and pollination distributions and variability. (A) Herbivory (solid line) and pollination (dashed line) distributions under predictable day-to-day variability. (B) An example of values representing high among-year variability ($\sigma^2 =$ 408.3, mean = 30) in herbivory timing across years.

RESULTS AND DISCUSSION

Results of my model show that annual plants experiencing stochastically variable herbivory and pollination (a bet-hedging scenario) should evolve allocation schedules switching abruptly to reproduction late in the season relative to plants experiencing no variability. Perennial plants experiencing stochastically variable herbivory and pollination should evolve gradual allocation schedules with allocation to reproduction beginning early in the season.

In my model, lack of variability in within-year herbivory and pollination peaks resulted in selection for a bang-bang allocation schedules for the annual and perennial plant (Figure 11). These patterns of schedules were favored over repeated runs of the model (data not shown).

Other models considering the absence of environmental variability also predict evolution of the bang-bang schedule in both annual plants (Cohen, 1971) and perennials with underground storage (Perrin and Sibly, 1993). The optimum time to begin allocating to reproduction for both annuals and perennials in my model likely reflects synchrony between flowering and pollination, since both the switch to reproductive allocation and peak pollination coincide at day 60. Pollination-flowering synchrony has been shown to be important for fitness in some plant species, for example *Erythronium grandiflorum* (Thomson, 2010).

When I allowed stochastic variability in the timing of peak herbivory and pollination, allocation schedules with later switches were favored in annual plants (Figures 12A-C). Other models incorporating variability in end-of-season predict that annuals will evolve a gradual allocation schedule (Cohen, 1971; King and Roughgarden, 1982) or a greater range of the timing of the switch (Satake *et al.*, 2001). The late switch to reproduction seen in my model seems risky, but variability in end-of-season that other models consider is more likely to punish late allocation schedules than variability in non-lethal herbivory, which will allow a plant to continue growth and reproduction even after disturbance.

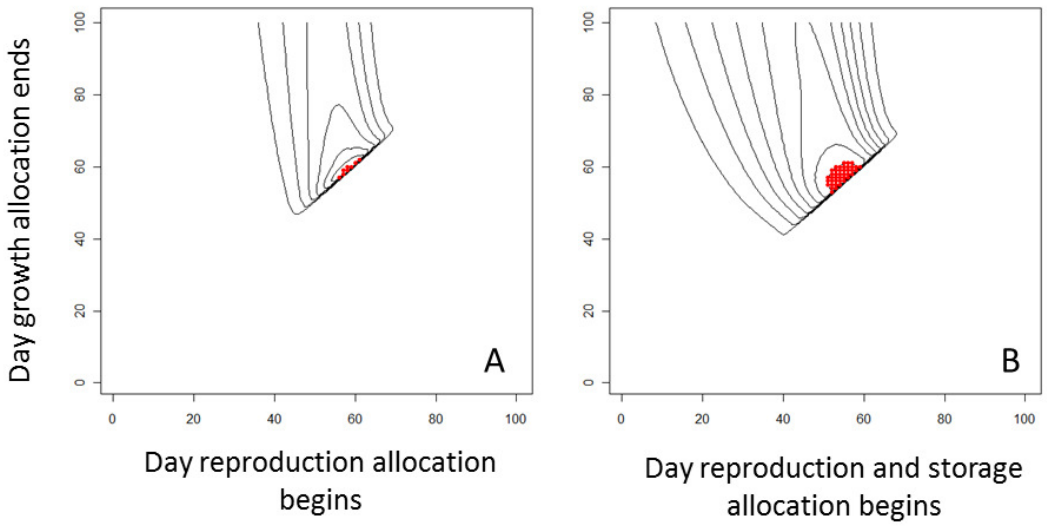

Figure 11. Fitness surfaces of annual and perennial plants with no variability in herbivory and pollination. Surfaces are responses of (A) an annual plant and (B) a perennial plant. Z axis is annual fruit set.

Additionally, my model includes variability in a factor beneficial to plant fitness: pollination. The late-flowering plants favored in this model can take advantage of a late peak in pollination after they have grown all season and can produce many flowers. Predictions of models with a single, acute disturbance may match many natural systems, for example in harsh environments where early-flowering annuals are common (e.g., the arctic, Totland, 1997). In systems where those predictions don't match, that is where late-flowering annuals are common, variability in herbivory and pollination might be more pronounced than variability in end-of-season. Empirical studies to support or refute such a hypothesis are lacking.

When stochastic variability in the timing of herbivory and pollination was added to my model, with increasing levels of variability, gradual allocation schedules were favored for perennial plants (Figures 13A-C). Stochastic variability in end-of-season in perennial plants is expected to lead to selection for a gradual transition between growth and flowering (Perrin and Sibly, 1993). My model demonstrates this result as well as a slightly more scattered range of favored allocation schedules (Figure 13A-C). Although I know of no examples of more gradual allocation schedules occurring in natural populations with more temporal variability in insect foragers, gradual schedules have been found for a number of perennial plant populations, including *Silene uniflora* (Pettersson, 1994) and *Lotus corniculatus* (Ollerton and Lack, 1998), and so the phenotype is certainly possible. There is agreement between my model and models considering variability in acute disturbances, such as end-of-season (Perrin and Sibly, 1993), about the evolution of gradual transitions. This suggests that the gradual schedule may be a general bet-hedging solution for perennial plants under a range of types of environmental variability. In my model, herbivory reduces plant size but does not cause mortality, an effect found in natural systems as well (Underwood and Halpern, 2012). Size-dependent mortality, where smaller plants experience more mortality than larger plants independent of herbivory, might select for longer periods of growth prior to allocating resources to reproduction (i.e., Perrin and Sibly, 1993).

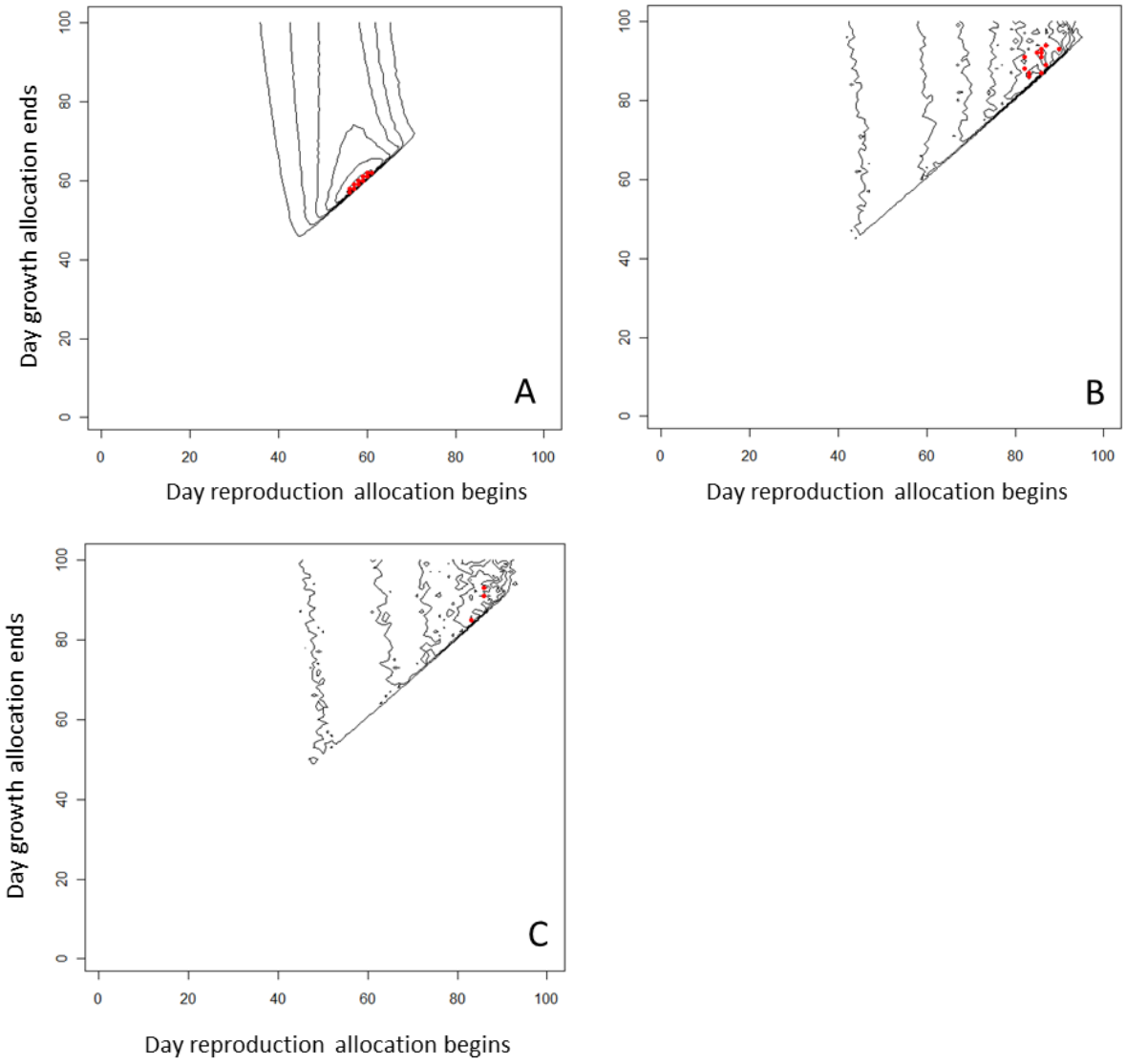

Figure 12. Fitness surfaces of annual plant. Variability in herbivory and pollination is (A) low, (B) intermediate, and (C) high. Z axis is annual fruit set. See Table 4 for variability values.

In my model, variability in the timing of herbivory and pollination are correlated (that is, when variability is high in one it is also high in the other, and vice versa) in order to address the joint effects of plant antagonists and mutualists on evolution of allocation schedule. Correlated variability is a reasonable condition for insect herbivores and pollinators that respond similarly to abiotic conditions like temperature (Gullan and Cranston, 2000). If variability in abiotic conditions is stochastic, as might increasingly be the case as the global climate changes (Schar *et al.*, 2004), stochastic variability in both herbivores and pollinators might follow. This is likely particularly true when the same species acts as both herbivore and pollinator, as is common in the Lepidoptera. Varying herbivory and pollination independently in the model would tell me the contributions of each to the evolution of allocation schedule. This might be important for herbivory and pollination by non-insect organisms, and a future version of this model will

include independently varying herbivory and pollination. Since herbivory and pollination influence fitness in different ways in this model (pollination directly influences fitness, while herbivory indirectly influences fitness through plant size), I would expect that pollination might be largely responsible for the location of the optimal allocation schedule, because if peak flower production coincides with pollination, fitness will benefit most directly. Herbivory on the other hand might be more responsible for the strength of selection for the optimum, since herbivory affects the total number of flowers a plant can produce and so might be more likely to influence the height of peak fitness.

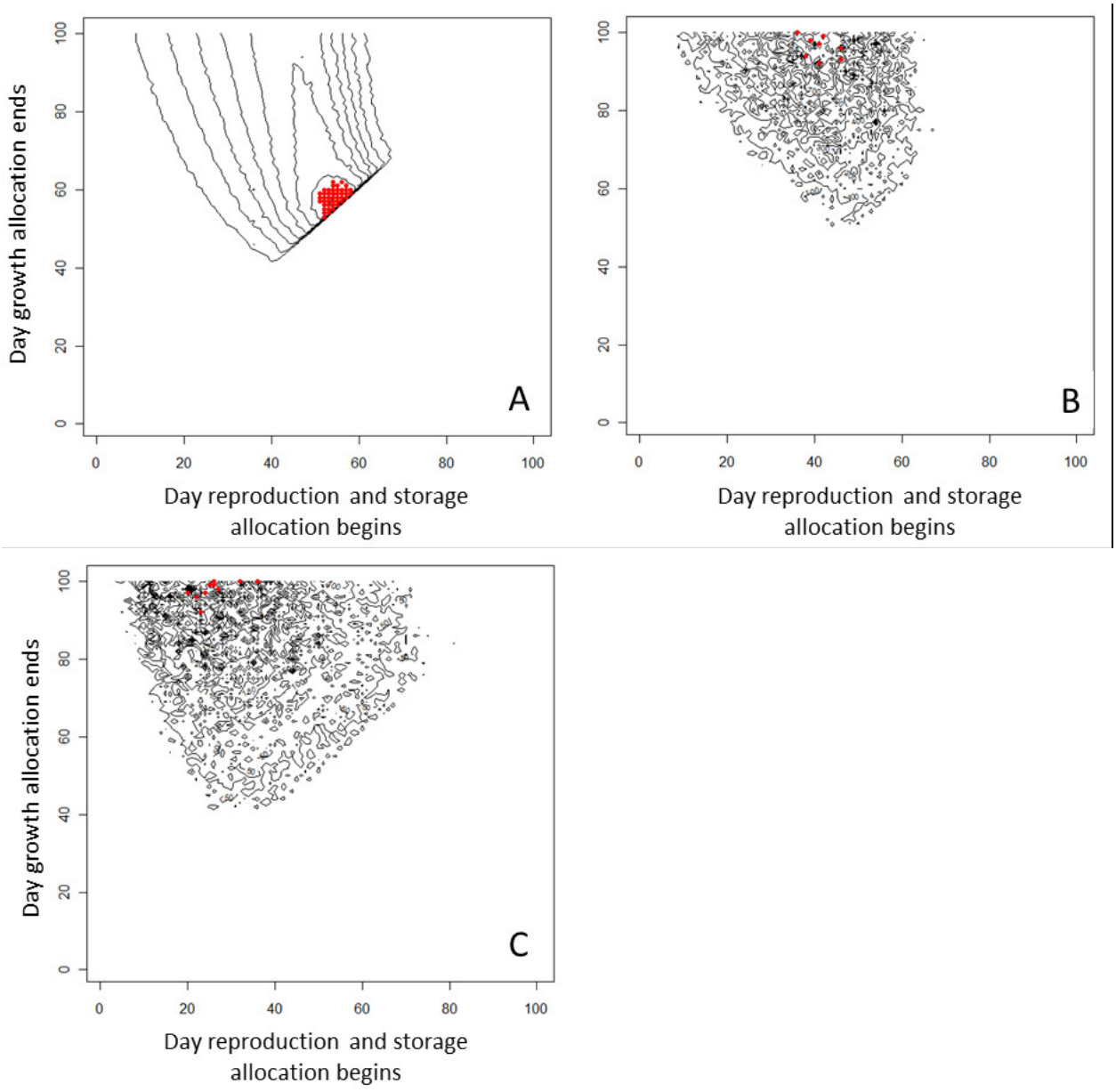

Figure 13. Fitness surfaces of perennial plant. Variability in herbivory and pollination is (A) low, (B) intermediate, and (C) high. Z axis is annual fruit set. See Table 4 for variability values.

The location of the peaks on the fitness surface indicates the optimal allocation schedule, but the steepness of the slope indicates how strongly that schedule will be favored. I have illustrated the steepness of the fitness surface by showing the area of the surface within 10% of the maximum value (in red in Figures 12 and 13). A smaller area within 10% of the maximum indicates stronger selection for that schedule, that is, a harsher fitness penalty for deviation from the maximum. The fitness penalties for deviation from the maximum are high in all the cases my model examined, as the peak area is never more than 1% of the available fitness surface (Figure 14).

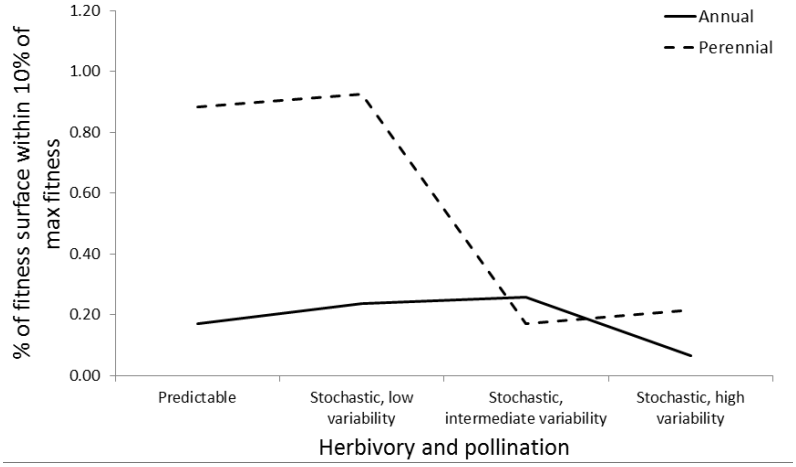

Figure 14. The percent of available fitness surface within 10% of the maximum. This is an indication of the sharpness of the peaks and strength of selection for the peak. As variability increases the area within 10% of the maximum decreases, reflecting stronger selection for those peaks.

CONCLUSIONS

This model produced predictions for the evolution of allocation schedule in a stochastically variable environment of both antagonists and mutualists. First, annual plants are predicted to evolve a later, but still bang-bang, allocation switch to reproduction relative to plants experiencing no variability, while other models of annual plants in variable environments predict the evolution of a more gradual allocation schedule (Cohen, 1971; King and Roughgarden, 1982). This difference could arise due to the nature of the variable factor: other models incorporate variability in an acute, lethal disturbance, while I incorporate a chronic sub-lethal disturbance (herbivory) accompanied by a chronic benefit (pollination). To my knowledge, empirical tests of variability in chronic sub-lethal antagonists and mutualists are lacking. Second, perennial plants are predicted to evolve gradual allocation schedules. My result is in general agreement with previous models incorporating variability in acute lethal disturbances. My results suggest that annual plants evolve different allocation schedules under different types of environmental variability, while perennial plants evolve a gradual allocation schedule under either lethal or sub-lethal disturbances.

CHAPTER FOUR

EFFECT OF DAMAGE AND POLLINATION ON PLANT MODE OF REPRODUCTION

INTRODUCTION

Plants invading a new range are faced with many challenges, among them avoiding herbivores and attracting pollinators in their new environment. Although escape from herbivores may be a characteristic of some successful plant invasions (Maron and Vila, 2001; Keane and Crawley, 2002), herbivores can be present in an invaded range. Herbivores may also be introduced to a plant's invaded range as a biocontrol method, for example with the cactus moth *Cactoblastis cactorum* introduction in Australia (Raghu and Walton, 2007), and the water hyacinth weevil *Neochetina* spp. introduction in North America (Center *et al.*, 1999b). Pollinators can influence plant invasion by increasing sexual fitness of the invaders (Brown *et al.*, 2002), although asexual reproduction or selfing can also allow invaders to reproduce without pollinators (Herrera and Nassar, 2009). How herbivores and pollinators influence traits of invasive plants, particularly reproductive traits associated with population spread and establishment might have consequences for management of plant invasions.

Herbivores and pollinators are known or suspected to influence both sexual and asexual reproductive traits in plants. Herbivore damage to leaves is known to reduce several aspects of sexual reproduction (e.g. number of pollen grains and pollen performance in *Cucurbita texana* (Quesada *et al.*, 1995), flower size in *Raphanus raphinistrum* (Lehtilä and Strauss, 1997) and *Impatiens capensis* (Steets and Ashman, 2004), and number of open flowers in *Datura wrightii* (Elle and Hare, 2002)). Some studies suggest that herbivory may be more likely to influence sexual reproduction than asexual reproduction, where plants maintain rhizome size (Meyer and Root, 1993; Wise *et al.*, 2006) or clonal reproduction (Bråthen and Junttila, 2006) after damage, even while sexual reproduction is compromised. However, herbivores may be able to influence asexual reproduction through plant size (Underwood and Halpern, 2012). For example, smaller plants may invest more in asexual reproduction relative to sexual reproduction (but see Verburg and Grava, 1998; Svenning, 2000; Aarssen, 2008). Herbivore damage is also known to influence plant defenses (Kaplan *et al.*, 2008), and if trade-offs between defense and reproduction exist (Karban, 2011), herbivores may be able to influence allocation to reproduction indirectly.

Pollination also has been shown to influence sexual reproductive traits, for example by decreasing the length of time that flowers are open in *Chamerion angustifolium* (Clark and Husband, 2007) and number of open flowers in *Satyrium longicauda* (Harder and Johnson, 2005). Pollination failure in early-opening flowers increased nectar volume and pollen receipt in late-opening flowers of *Alstroemeria aurea* (Ladio and Aizen, 1999), and caused a shift from semelparity to iteroparity in *Ipomopsis aggregata* (Paige and Whitham, 1987). While there is to my knowledge no study exploring the effect of pollinator availability on clonal plant traits, pollination has been show to affect plant size traits (Snow and Whigham, 1989) which could in turn influence asexual reproduction. In addition, tradeoffs between asexual and sexual reproduction (e.g., Geber, 1990; Bowers, 1996) could implicate pollinators in indirect effects on asexual reproduction.

Because mode of reproduction can have important implications for dispersal ability and offspring establishment, herbivore and pollinator effects on mode of reproduction could be

important for spread of establishment of invasive plant species. Sexual reproduction allows for longer-distance seed dispersal (Eriksson, 1997), while asexual reproduction through vegetative propagation might confer high rates of offspring establishment and survival (Herrera and Nassar, 2009). Knowing whether a plant species is likely to disperse or establish easily may be important for management: easily dispersing species may need strong quarantine measures while easily establishing species may need local population suppression measures. If a plant's biotic environment (its herbivores and pollinators) influences that plant's mode of reproduction, those responses need to be taken into account in invasive plant management.

Here I use the invasive aquatic plant *Eichhornia crassipes* to look at how pollination and damage by different life stages of a specialist weevil influence investment in flowers and clonal offspring. Larval weevils damage apical and axillary meristems while adult weevils damage leaves. I expect apical meristem damage (i.e., loss of sexual reproduction) to cause a shift to asexual reproduction, while I expect axillary meristem damage (i.e., loss of asexual reproduction) to cause a shift to sexual reproduction. If leaf damage is more detrimental to sexual than asexual reproduction, as has been suggested in some studies (Meyer and Root, 1993; Bråthen and Junttila, 2006; Wise *et al.*, 2006), I expect leaf damage to reduce flowering and either increase or cause no change in clonal offspring production.

In order to assess the potential contribution of induced resistance to investment in sexual and asexual reproduction, I also test for induced resistance to manual and insect damage. To assess natural levels of reproductive expression, damage, and resistance in natural *E. crassipes* populations, I conduct population surveys and common garden experiments.

MATERIALS AND METHODS

Study System

Eichhornia crassipes (water hyacinth, Pontederiaceae) is a freshwater aquatic perennial native to South America. Plants float freely in rivers and lakes, the roots and central rhizome buoyed by air-filled petioles. An apical meristem produces new leaves up until an inflorescence is formed, after which no new leaves can be produced. Axillary buds are formed with each new leaf, and can either produce clonal daughter plants via stolons, or less often, can produce a continuation stem that allows the parent plant to produce an additional inflorescence (Geber *et al.*, 1992). Inflorescences have many lavender flowers (mean inflorescence number = 6.3, SE = 0.3 in this study, n = 62), which are pollinated by a variety of insect visitors in the native range (Barrett, 1980). Flowers are self-compatible but herkogamy and a lack of pollinators seem to limit seed set in the north Florida populations used in this study (pers obs). Plants were collected from five populations in north Florida (Figure 15): Lake Munson (N30.37000°, W84.31465°; hereafter "MUN"), the upper Wacissa River (N30.34384°, W83.99923°; hereafter "WAC"), the Crooked River north of Carabelle, FL (N29.92872°, W84.62568°; hereafter "CRK"), the St. Marks River north of Newport, FL (N30.225978°, W84.156243°; hereafter "STM"), and a canal at the confluence of the St. Marks and Wakulla Rivers in St. Marks, FL (N30.155261°, W84.208277°, hereafter "SMW").

Figure 15. Map of five *Eichhornia crassipes* populations sampled in north Florida. Survey and collection sites are marked with a solid circle (•). Experiments took place at the Mission Road greenhouse facility in Tallahassee.

The specialist water hyacinth weevils *Neochetina eichhorniae* and *N. bruchi* (Curculionidae) are used for biocontrol in the US. Both species have been found to reduce *E. crassipes* size and inflorescence production (Center *et al.*, 2005) as well as population growth (Center *et al.*, 1999b). These two species were used interchangeably in the experiments described here. Weevils were collected from plants at the STM site and at Weems Pond in Tallahassee, FL (marked with * in Figure 15, N30.455525°, W84.222849°).

The experiments for this study were conducted in spring and summer of 2010, 2011, and 2012 (see Table 5 for summary of experiments), at the Florida State University Mission Road facility (N30.458269°, W84.332954°) in either outdoor cattle tanks (325L) or indoor greenhouse glass tanks (50L). New plants were provided for each set of experiments. Plants were supplied with well water in all years, and in 2011 and 2012 were fertilized as needed with 2-4 g/L of Suncote 15-8-11 slow release fertilizer and 0.2 g/L Miller Iron Chelate DP 10%Fe. Herbivores were removed from plants in outdoor tanks as they were found, but some caterpillar feeding damage (estimated < 1%) occurred. No pollinators were observed at any point.

Direct influence of damage on flowers and clonal offspring

I conducted similar experiments in 2010 and 2011 to examine the effects of four damage treatments on *E. crassipes* allocation to flowers versus clonal offspring. In the 2011 experiment I also manipulated pollination. Damage treatments in both experiments simulated larval or adult weevil damage by manually imposing damage. Damage treatments were intended to investigate how different damage types, not necessarily damage amount, influenced plant responses. Weevil

larvae damage both apical and axillary meristem tissue (Stark and Goyer, 1983; Center *et al.*, 2005). To simulate larval damage to apical meristems ("AP" damage treatment) I used forceps to destroy the single apical meristem of the plant by removing the youngest unexpanded leaf and the underlying tissue down to the rhizome. To simulate larval damage to axillary buds ("AX" damage treatment) I used forceps to destroy all visible axillary buds and underlying tissue down to the rhizome, terminating stolon production. Adult weevils of both species leave characteristic feeding scars on the leaf surface. To mimic adult damage I used a razor blade to remove approximately 25-30% of the plant's leaf surface tissue evenly distributed across expanded leaves ("LF" damage treatment) in small patches while leaving underlying fibrous tissue. Manual leaf scarring was three times more severe than the maximum damage by adult weevils in the natural populations surveyed (approximately 10% of leaf area, pers. obs). Leaf damage was severe in these experiments to assess potential plant responses to considerable damage. Control plants ("CN") were not damaged, but were handled similarly to damaged plants to account for effects of physical manipulation.

Although the 2010 and 2011 experiments used identical damage manipulations and measured responses, there were some differences in experimental design. In 2010, AX damage was performed once, while in 2011 AX damage was ongoing for ten days. Experiments differed in sample size (n = 120 in 2010, n = 306 in 2011), exposure to insect herbivores (plants in all damage treatments were exposed to weevils in 2010, while no plants were exposed to weevils in 2011), and time frame of measured responses (allocation responses were measured 37 days after damage in 2010, and 11 days after damage in 2011, plant survival was also measured through 100 days after damage in 2011). The 2011 experiments also included pollination treatments, with pollen receipt (hand pollination or no pollination) crossed with all four damage treatments.

Allocation response to manual damage: 2010

In 2010, 120 plants (38 CRK, 8 MUN, and 74 WAC; population representation is uneven due to population size differences) were maintained in six outdoor tanks, each holding 20 plants. I measured initial leaf number and wet biomass before imposing one of the four damage treatments, LF, AP, AX, or CN, distributed evenly across tanks and populations. I measured change in leaf number from the initial measurement to 37 days post-damage. I measured number of clonal offspring produced as clonal offspring at 37 days post-damage (all plants began the experiment with no clonal offspring). Plants were monitored for flower production but almost no plants flowered in this experiment.

Net change in leaf number and total clonal offspring number (log transformed) were analyzed with separate Type III SS ANOVAs. Original models included damage treatment, population of origin, tank, and all interactions. Tank and population were treated as fixed factors, because multiple populations were sampled only for genetic diversity, not to investigate population differences, and because I am not concerned with interpreting effects of identity of tank. I conducted hierarchical model reduction by eliminating non-significant interactions with a drop function (R Development Core Team, 2008). Where ANOVA yielded significant effects, I performed Tukey's HSD for pairwise comparisons between damage treatments. The final model for change in leaf number included damage treatment, population, and tank main effects; the final model for clonal offspring number included damage treatment.

Table 5. Experiments performed in 2010, 2011, and 2012 on *Eichhornia crassipes*. Responses indicate those measured, not necessarily those included in the final model of the analysis. See text for details.

RESPONSE VARIABLES	PREDICTOR VARIABLES	YEAR
Direct influence of damage on flowers and clonal offspring		
Allocation response to manual damage		2010
Leaf number	Manual weevil damage (apical, axillary, leaf, control)	
Clonal offspring number	Population	
	Block	
Allocation response to manual damage and pollination		2011
Leaf number	Manual weevil damage (apical, axillary, leaf, control)	
Clonal offspring number	Pollination (supplemental, none)	
Flowers produced	Population	
Plant survival	Initial mass	
Fruit number	Initial leaf number	
Seed mass	Block	
Allocation response to insect damage		2012
Leaf number	Leaf damage (manual, insect, control)	
Clonal offspring number	Initial leaf number	
Indirect influence of damage via induced resistance		
Defense response to manual damage: whole-plant choice		2010
Scar number	Manual weevil damage (apical, axillary, leaf, control)	
Number of weevils per plant	Block	
Defense response to manual damage: no choice bioassay		2011
Scar area	Manual weevil damage (apical, axillary, leaf, control)	
	Date of bioassay	
	Population	
	Block	
Defense response to adult weevil damage: no-choice bioassay		2010
Scar number	Leaf damage (manual, weevil, control)	
	Block	
Defense response to adult weevil damage: whole-plant choice		2010
Number of weevils per plant	Leaf damage (manual, weevil, control)	
Natural levels of reproductive expression, damage, and resistance: field surveys		2011
Plant density	Population	
Leaf number		
Clonal offspring number		
Weevil density		
Inflorescence density		
Genetic variation in reproduction and defense: common garden		2011
Leaf number	Population	
Clonal offspring number		
Flowers produced		
Bioassay scar area		

Allocation response to manual damage and pollination: 2011

In 2011, 306 plants (153 WAC and153 CRK) were maintained in 38 greenhouse tanks, each tank containing four plants from each population. I measured initial leaf number and plant mass, and imposed each one of the four damage treatments evenly across tanks and populations. LF and AP treatments took four days to impose on all 306 plants, while AX treatments continued over 14 days as new buds developed. Data collection subsequent to initial damage occurred over four days so that data were collected from all plants after the same time since damage. After 14

days, plants became too large for their tanks and were transferred to outdoor cattle tanks with approximately 20 plants per tank.

I measured allocation responses to damage 11 days after initial damage, after which high and unbalanced mortality, particularly affecting AP plants, allowed analysis of survival data only. High mortality in AP plants was likely due to damage treatments, but mortality across damage treatments may have been due to a failure to thrive in the indoor tanks. Survival was assessed at 27, 41, 60, 80, and 100 days post-damage. Fruit number was measured in plants receiving hand pollination (no fruit set occurred without hand pollination). Fruits were collected as they matured, and seeds were separated from fruits and weighed.

I analyzed change in leaf number and total clonal offspring number 11 days after damage treatment with Type III SS analysis of covariance (ANCOVA). Models included damage treatment, initial leaf number (except where change in leaf number is the response), initial plant mass, tank, and all two-way interactions (except tank) with damage treatment. I performed model reduction as for the 2010 data. Flower production and survival to 27, 41, 60, 80, and 100 days were analyzed with Chi-squared tests. Fruit set (per pollinated flowers) and seed mass per fruit for hand-pollinated plants were analyzed with Type III SS ANOVA, including damage treatment, population, and damage-by-population interaction. Final models included only main effects of damage treatment and population.

As plants in the 2011 damage experiment began to develop flowers (between days four and 11 after initial damage), they were assigned to either hand pollination or no pollination treatments, so that each damage treatment group had approximately equal numbers of pollinated and non-pollinated plants (except the apical damage group, which did not produce flowers). Pollination treatments were imposed on 64 plants (31 hand pollinated, 33 not pollinated) using pollen from a different population.

The effect of pollination treatment was analyzed for the difference in leaf number from the start of the experiment (day zero) and day 27 (at least two weeks after pollination treatments ended), and square-root transformed total clonal offspring production from day 11 (near the end of pollination treatments) to day 27. Lack of subsequent flower production precluded further analysis of flowering response.

To analyze pollination main effects and damage-by-pollination interactions I used type III ANCOVA models including pollination treatment, damage treatment, initial leaf number (except where leaf number is the response variable), initial plants mass, and tank. Because this analysis is intended to examine the damage by pollination interaction, the interaction term was always retained in the model.

Allocation response to insect damage: 2012

In the experiments described above I used manual damage to assess allocation responses because this allowed more experimental control, but insect damage may differ in consequences from manual damage. In order to determine if insect herbivory alters allocation to leaves, clonal offspring, or flowers, I tested allocation responses to adult weevil (leaf) damage, manual leaf damage, and no damage (control). Difficulties in manipulating larval weevils prevented me from testing allocation responses to weevil meristem damage. Sixty plants (SMW) were maintained in a single outdoor tank. Twenty plants were individually bagged with two male weevils (to prevent oviposition and larval damage) for four days; control and manually damaged plants were bagged but undamaged for this period. Insect damage in this experiment averaged about 3% of the leaf surface, within the natural range of damage. After four days, weevils were removed and manual

damage was imposed on twenty different plants. Manual damage was imposed as in the previously described experiments at approximately equal levels of that imposed by insects in this experiment (3-4%). Leaf, clonal offspring, and flower production were measured at seven, 14, 21, and 28 days post-damage. I analyzed damage treatment effects on log-transformed change in leaf number and log-transformed total clonal offspring production with initial leaf number as a covariate with Type III SS ANOVA. Only one plant produced flowers during this period, so flower production was not analyzed.

Indirect influence of damage via induced resistance

Damage treatments could influence plant allocation patterns directly, but induced defenses in response to damage (Kaplan *et al.*, 2008) could also affect allocation to reproduction through a predicted cost of defense (Stamp, 2003). Although simulated damage may fail to elicit the same responses as real herbivory (Walling, 2000), manual damage can induce defenses in some cases (McCall, 2006). I conducted experiments in 2010 and 2011 to assess *E. crassipes'* defense response to manual and insect damage, measured by adult beetle, no-choice feeding bioassay (in 2011) and whole plant choice trials (in 2010). Insect preferences at the level of whole-plants would indicate induced, but not necessarily systemic, defenses in response to the four manual damage treatments. Insect responses to undamaged leaves in the no-choice bioassay would indicate induced systemic defenses in response to the four manual damage treatments.

Defense response to manual damage: whole-plant choice

This experiment used the 120 plants in the 2010 manual damage experiment (38 CRK, 8 MUN, and 74 WAC). Sixty to 72 hours after damage treatments, the plants were split into groups of ten (two per outdoor tank) approximately evenly across the four manual damage treatments (AP, AX, LF, and CN) and population of origin. The number of scars from manual damage was counted, and each group of ten plants was placed in a large mesh bag. Twenty *Neochetina* spp. weevils were released into half the groups, where they quickly found refuge in the plants and were allowed to forage for four days. After four days, I noted which plants weevils were on, removed them from the plants, and counted scars on the leaves. I quantified herbivore choice as weevil number per plant and amount of weevil damage per plant (difference in scar number before and after weevil introduction). I repeated these procedures again 12 days after initial damage treatments, reversing herbivore and no-herbivore treatments so that previously unexposed plants received herbivores.

I analyzed the effect of initial damage treatment on the change in scar number (log transformed) accumulated over both rounds of damage with a fixed effects Type III SS ANOVA, with tank as a blocking factor. I analyzed effect of damage treatment on number of herbivores recovered from plants from the early-exposure group (log transformed) and the late exposure group (square-root transformed) separately, with Type III SS ANOVAs. Variables were transformed to meet assumptions of normality and homogeneity of variance.

Defense response to manual damage: no choice bioassay

This experiment used a subset of plants from each damage treatment in the 2011 damage experiment. Induced response to manual damage type was measured with a bioassay two days after damage (n = 109 plants, minimum 20 per damage treatment) and ten days after damage (n = 80 plants, minimum 18 per damage treatment). I excised and photographed the central expanded leaf of each plant and placed the leaf in a 5.5 ounce (163 mL) plastic cup, filled with water and a

paper towel to elevate the leaf blade above the water, enclosed in a mesh bag. Two to three adult *Neochetina* spp. weevils were allowed to feed for 24 hours, after which the leaf was re-photographed. Photographs were analyzed to measure leaf area damaged during the bioassay. Scar area per weevil was used as a measure of susceptibility to herbivores in response to different damage types. Scar area was analyzed with Type III SS ANOVA in response to damage treatment, date of bioassay, population, and tank main effects. All factors were fixed because multiple populations were sampled only for genetic diversity, not to investigate population differences, and because I am not concerned with interpreting effects of identity of tank.

Defense response to adult weevil damage: no-choice and whole-plant choice

In 2010 I tested for induced resistance following insect damage. Thirty plants (from population CRK) received either manual damage, insect damage, or no damage, and plant resistance after damage was measured in no-choice and choice bioassays. Manual damage was imposed as in the previous experiments, damaging approximately 25-30% of the leaf area. Leaf damage was imposed by bagging an entire plant and allowing 10-15 weevils per plant to feed for four days. Final damage levels were approximately 10% damage per leaf. Weevils were not sexed, so females may have had the opportunity to lay eggs, but eggs would not have had time to hatch before the bioassay (Deloach and Cordo, 1976; Stark and Goyer, 1983) so damage was by adults only. Induced resistance to larval damage was not measured due to difficulty in manipulating larvae, which mostly feed internally. Control plants were handled but not damaged. Three plants (one from each damage treatment) were caged in each of ten water-filled trays in the greenhouse, so that each cage formed a three-plant block.

For the no-choice bioassay, I excised the central expanded leaf of each plant for an overnight 14-hour no-choice bioassay by four weevils. The level of plant resistance was measured as the difference in scars on each leaf before and after damage. The difference in the number of scars (square-root transformed) was analyzed with a Type III SS ANOVA in response to damage treatment and cage (fixed factors) main effects. The final model included damage treatment only.

For the whole-plant choice, I left the plants in the three-plant caged blocks and introduced 10-15 randomly selected weevils from the previous feeding trials. The weevils were allowed to forage for 24 hours. I used a Type III SS ANOVA to analyze proportion of weevils found on each plant in response to damage treatment type, with plant biomass as a covariate.

Population surveys

Natural levels of reproductive expression, damage, and resistance: field surveys

To obtain information about levels of herbivory and allocation in natural populations of *E. crassipes*, on June 24-26, 2011, I surveyed three north Florida *E. crassipes* populations (CRK, WAC, and STM). I measured plant density and clonal offspring density in 0.5 x 0.5 m quadrats, leaf number per individual per quadrat, and weevil density per plant with a visual search. Population differences in plant density, leaf number, clonal offspring number, weevil density, and inflorescence density recorded in the field were analyzed with a single factor multivariate analysis of variance (MANOVA). Post-hoc ANOVAs were performed if MANOVA results indicated significant differences. I excised and photographed three haphazardly chosen leaves from a minimum of 20 plants in each population to measure leaf size and damage via image analysis; differences among populations were analyzed with ANOVA.

Effects of population were also included in the 2010 and 2011 damage and pollination experiments. For clarity I will discuss any significant effects of population on leaf number, clonal offspring production, or herbivore preference from those experiments in conjunction with population differences found in field surveys.

Genetic variation in reproduction and defense: common garden

To determine if differences in allocation pattern or resistance among populations might have a genetic basis, I transplanted plants from each surveyed population into a common environment. In 2011, I collected 20-24 individual plants from each of three populations (CRK, WAC, and STM), selecting apparently representative individuals throughout each population. Plants from each population were split evenly between two outdoor cattle tanks and surveyed for leaves, clonal offspring, and flowers immediately after transplant (day 0), and again on days 17, 30, and 37 after transplant. Due to rapid asexual reproduction by focal plants, clonal daughter plants were removed from parent plants at each survey to prevent them from breaking off and becoming untraceable to their parent. Bioassays were performed at day 20 and day 36, on a different subset of plants each time. For each bioassay, the central expanded leaf of each parent plant was excised, photographed front and back, and placed in a bioassay chamber with three *Neochetina* spp. adults for 24 hours, and re-photographed for image analysis of damaged area using ImageJ measurement software (Rasband 2012).

Population differences in leaf number at day 16 and day 53 after transplant of the common garden experiment were analyzed with two separate Type III SS ANOVAs. Population differences in clonal offspring number were analyzed with two separate Kruskal-Wallis tests. Population differences in flower and inflorescence presence/absence were each analyzed with a single binomial GLM including only the end of the experiment, since no flowers or inflorescences were present at the beginning. Each model for leaf number, clonal offspring presence, and inflorescence presence included only population as the independent variable. Population effects on leaf area damaged during bioassays at day16 and day 33 after transplant were analyzed with a Type III SS ANOVA including only population as the independent variable. All analyses were done R 2.10 (R Development Core Team, 2008).

RESULTS

Direct influence of damage on flowers and clonal offspring

In 2010, plants with apical damage produced fewer leaves than plants in all other damage treatment groups ($F_{3,109} = 12.56$, $P < 0.0001$, Table 6, Figure 16A) and produced more new clonal offspring relative to both control and leaf-damaged plants ($F_{3,116} = 3.77$, $P = 0.012$, Figure 16C). In 2011, plants with apical damage produced fewer leaves than control plants ($F_{3, 262} = 108.39$, $P < 0.0001$, Figure 16B), and in 2011 were less likely to flower over the entire 100 day experiment ($\chi^2 = 24.23$, df = 3, $P < 0.0001$; Figure 17). In 2011, axillary damage increased leaf number ($F_{3, 262} = 108.39$, $P < 0.0001$, Figure 16A) and decreased mean clonal offspring number ($F_{3, 299} = 12.71$, $P < 0.0001$, Figure 16C). Damage type also influenced mortality in 2011 ($\chi^2 = 32.68$, df = 12, $P = 0.001$; Figure 18), with plants receiving apical damage not surviving as long as plants in other damage treatment groups. No damage treatment influenced fruit set or seed mass. In no experiment did leaf damage affect leaf or clonal offspring production. In 2012, insect damage had no effect on leaf ($F_{2, 56} = 0.99$, $P = 0.38$) or clonal offspring production ($F_{2, 55} = 0.22$, $P = 0.81$). Pollination treatments (2011 only) had no effect on leaf or clone production (data not shown).

Table 6. ANOVA table of responses to damage treatments in 2010 and 2011. These are final models reduced from original models (see text for details) by drop function in R.

Final model	Response	df	F	*P*
Change in leaf number (2010) = Damage treatment + population + block	Damage treatment	3	12.56	**<0.001**
	Population	2	8.35	**0.0005**
	Block	5	2.41	**0.04**
	Residual	109		
Change in leaf number (2011) = Damage treatment + initial mass + block	Damage treatment	3	108.39	**<0.001**
	Initial mass	1	15.68	**<0.001**
	Block	37	2.1	**0.0005**
	Residual	262		
Clonal offspring produced (2010) = Damage treatment	Damage treatment	3	3.77	**0.01**
	Residual	116		
Clonal offspring produced (2011) = Damage treatment + population	Damage treatment	3	12.72	**<0.001**
	Population	1	6.11	**0.014**
	Residual	299		

Indirect influence of damage via induced resistance

In 2010 whole-plant weevil choice experiments, leaf damage decreased damage received 37 days later ($F_{3,114} = 4.34$, $P < 0.01$, Figure 19A). None of the damage treatments affected number of herbivores recovered at either three or 12 days post-damage. None of the damage treatments differed significantly from undamaged plants in bioassay scar area ten days after damage. However, apically damaged plants and leaf damaged plants received slightly more damage than axillary damaged plants ($F_{3, 183} = 3.61$, $P = 0.02$, Figure 19B).

In bioassay experiments following manual and insect leaf-only damage, insect damage decreased subsequent damage ($F_{2,27} = 7.25$, $P < 0.01$, Figure 20) and increased variation in damage (Bartlett's K = 15.62, df = 2, $P = 0.0004$), but manual leaf damage had no effect on damage relative to control plants. Neither insect nor manual leaf damage influenced whole-plant herbivore choice ($F_{2, 24} = 0.9149$, $P = 0.41$).

Population surveys

Reproductive expression, damage, and resistance: field surveys

Populations differed in plant and herbivore characteristics in 2011 field surveys ($F_{2, 10} = 3.55$, P = 0.02). Post-hoc ANOVAs (Table 7) show differences in scar area per leaf area, leaf size, leaf number per plant, plant density per quadrat, and clonal offspring density per quadrat.

Population sometimes influenced plant traits in the 2010 and 2011 damage and pollination experiments. Change in leaf number in 2010 was largest in plants from CRK population (mean change in leaf number = 3.3, SE = 0.5) and smallest in WAC population (1.08 ± 0.1, effect of population $F_{2,109} = 8.35$, $P = 0.0004$). Clonal offspring production in 2011 was greater in plants from CRK (mean clonal offspring = 0.74, SE = 0.13) than from WAC (1.2 ± 0.15, effect of population $F_{1,299} = 6.12$, $P = 0.014$). In 2010, there were more scars received and more weevils found on plants from the MUN population (mean scar number = 161, SE = 17.6; mean weevil number = 1.25, SE = 0.78) than either the CRK (57.5 ± 8.4 and 0.16 ± 0.08, respectively) or WAC (68.3 ± 6.2 and 0.92 ± 0.15 respectively) populations ($F_{2,114} = 7.56$, P = 0.0008 and $F_{2, 54} = 5.62$, $P = 0.008$, respectively).

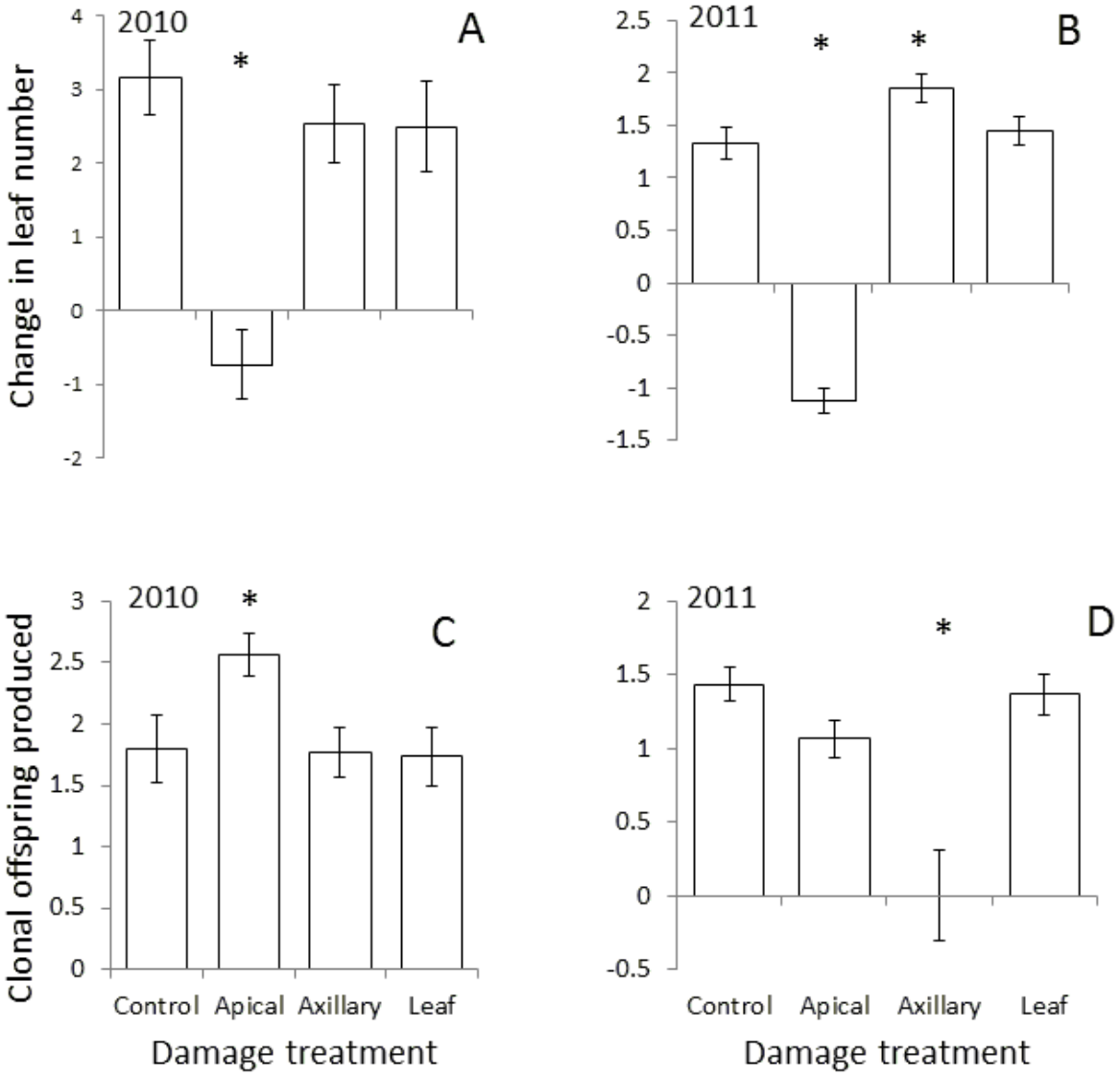

Figure 16. Responses to damage treatments in 2010 and 2011 experiments. * indicates significant (Tukey's HSD $P < 0.05$) difference from control; error bars show ± 1 SE. Mean change in leaf number in (A) 2010 and (B) 2011. Mean number of new clonal offspring produced in (C) 2010 and (D) 2011.

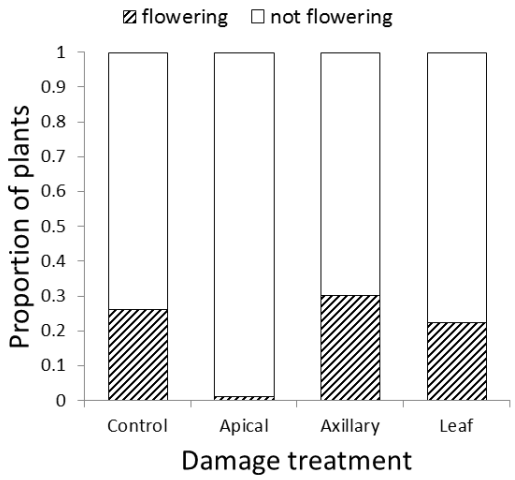

Figure 17. Apical damage decreased likelihood of flowering (χ^2 = 24.23, df = 3, P < 0.0001).

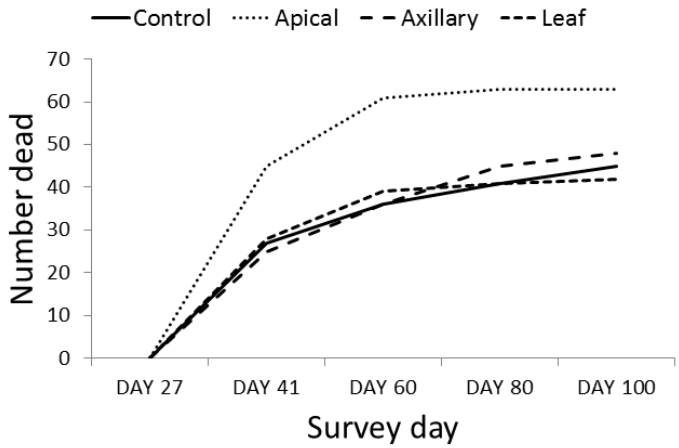

Figure 18. Cumulative mortality at each survey date in each damage treatment group. Damage type influenced survival time (χ^2 = 32.68, df = 12, P = 0.001).

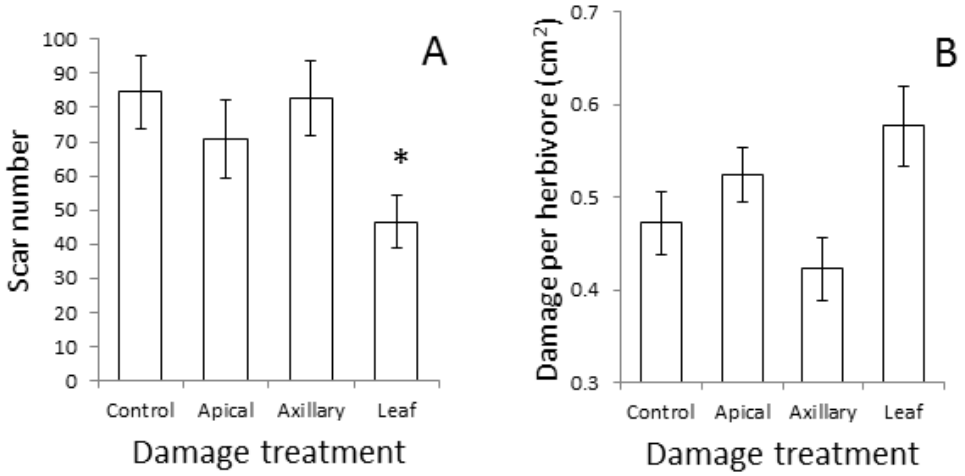

Figure 19. Herbivore response to damage. (A) Manual leaf damage reduced subsequent herbivore damage in whole-plant choice experiments (2010) relative to control plants. (B) Manual damage had no effect on subsequent damage in bioassay experiments (2011) relative to control plants. Leaf damaged plants received more damage than axillary damaged plants (Tukey's HSD $P = 0.02$). * indicates significant (Tukey's HSD $P < 0.05$) difference from control; error bars show ± 1 SE.

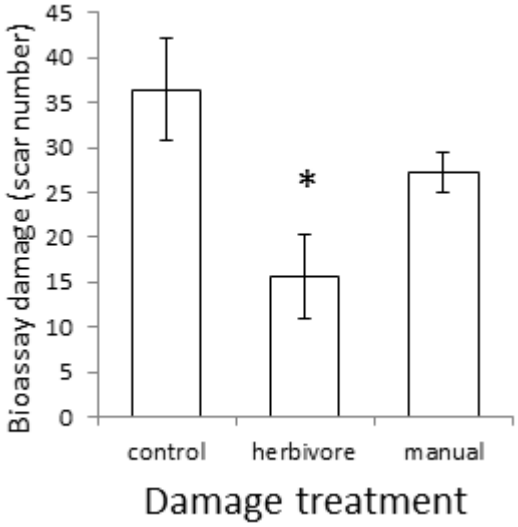

Figure 20. Induced resistance response to manual and herbivore damage. Plants induce resistance (measured as scar number) to herbivore leaf damage but not to manual leaf damage. * indicates significant (Tukey's HSD $P < 0.05$) difference from control; error bars show ± 1 SE.

Table 7. Plant traits and herbivory levels in three natural *Eichhornia crassipes* populations. Populations differed significantly where values are in bold. F- and p-values are from post-hoc ANOVAs.

Pop	% damage	Weevil density	Leaf number	Leaf area (cm^2)	Plant density (per 0.25 m^2)	Clone density (per 0.25 m^2)
WAC	0.04±0.02	0	5.02±0.23	19.25±1.82	1.4±0.4	0
CRK	5.7±2.0	3.25±2.63	7.4±0.05	41.79±3.5	6±0.58	3.5±1.04
STM	8.1±1.6	4±1.78	5.05±0.15	35.45±3.74	3.5±0.5	1.75±.48
F-value	11.96	1.7208	7.92	17.03	23.05	13.66
p-value	**< 0.001**	0.23	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.01**
df (res)	51	10	10	51	10	10

Genetic variation in reproduction and defense: common garden

Populations differed significantly in leaf number 16 days after transplant into a common garden ($F_{2, 62} = 4.429$, $P = 0.01$), but did not differ 53 days after transplant (Figure 21). Populations differed in clonal offspring number both at the beginning (Kruskal-Wallis $\chi^2 = 24.4953$, df = 2, $P < 0.0001$) and end (Kruskal-Wallis $\chi^2 = 6.5749$, df = 2, $P = 0.037$, Figure 22) of the common garden experiment, but the nature of those differences changed over the course of the experiment. At the early survey CRK had more clonal offspring than either STM or WAC populations. By the late survey, CRK and WAC had converged, while STM clonal offspring production rate remained low.

All populations produced inflorescences only at the later survey dates, and there were no significant population differences in inflorescence production 53 days after transplant. Populations differed in bioassay damage 33 days after transplant ($F_{2,26} = 4.5369$, $P = 0.02$). The CRK population received more damage (mean scars per leaf = 2.11, SE = 0.14) than either WAC (1.45 ± 0.17) or STM (1.39 ± 0.18) at the 33 day bioassay.

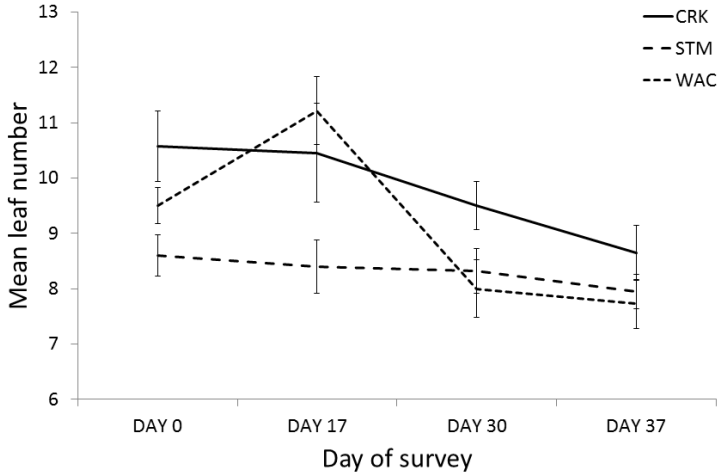

Figure 21. Mean leaf number for three populations over 37 days in a common garden. Populations differed in leaf number at day zero ($F_{2, 62} = 4.43$, $P = 0.01$) but not at day 37. Error bars show ± 1 SE.

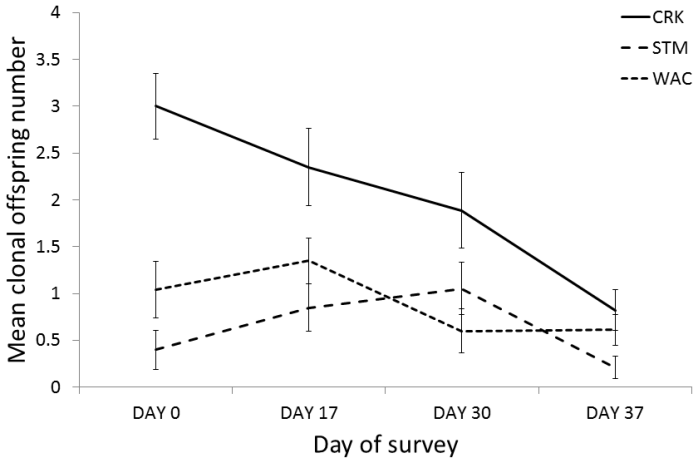

Figure 22. Mean clonal offspring number for three populations over 37 days in a common garden. Populations differed in clonal offspring number at day 0 ($\chi^2 = 24.4953$, df = 2, $P < 0.0001$) and at day 37 ($\chi^2 = 6.5749$, df = 2, $P = 0.03$), but values appear to be converging at the last survey. Error bars show ± 1 SE.

DISCUSSION

Plants invading a new range may face a biotic environment that influences their reproductive mode, and therefore their dispersal and establishment abilities. In this study, I asked how the invasive plant *Eichhornia crassipes* responds to damage and pollination. I found that (1) manual larval damage influenced reproductive expression directly, but manual adult damage had no effect; (2) manual larval and adult damage did not induce defenses (although real adult damage did), and therefore did not influence reproductive expression indirectly through allocation to defense; (3) populations differ in reproductive expression, damage, and resistance; but (4) there is little evidence for genetic differentiation in allocation to reproduction or defense among natural populations. My results suggest that *Neochetina* spp. larval herbivory may increase individual plant size via leaf production and increase population growth via promotion of asexual reproduction. Because these weevil species are frequently used for biocontrol, management of invasive *E. crassipes* populations should involve careful monitoring of *Neochetina* spp. effects.

Direct influence of damage on flowers and clonal offspring

Damage-induced resource allocation shifts are expected to preserve or even increase asexual reproduction, sometimes at the expense of sexual reproduction (Meyer and Root, 1993; Bråthen and Junttila, 2006; Wise *et al.*, 2006). My study seems to agree with these expectations, in that both apical and axillary meristem damage elicited allocation shifts toward plant parts that either directly increase asexual reproduction or may indirectly increase asexual reproduction. Apical meristem damage decreased leaf (Figure 16A-B) and inflorescence (Figure 17) production and increased clonal offspring production (Figure 16D). This suggests that inhibition of individual growth or sexual reproduction, or both, initiates increased allocation to asexual reproduction. An increase in asexual reproduction after apical damage in *E. crassipes* was

evident after 37 days (Figure 16B), but not after 11 days, indicating that while the effect is not immediate, in a species like *E. crassipes* that experiences rapid population growth due to asexual reproduction, allocation shifts to asexual reproduction can be reasonably swift.

Axillary meristem damage increased leaf production (Figure 16A) and did not change flower production. This suggests that inhibition of asexual reproduction initiates increased allocation to individual growth, but does not directly influence sexual reproduction. Because new leaves make possible new axillary buds (Geber *et al.*, 1992), an increase in leaf number following axillary damage could indirectly promote further clonal offspring production. An increase in clonal offspring production following an increase in leaf production would have been missed by this study (which measured clonal offspring production only to 37 days after damage) if new clonal offspring production lagged substantially behind leaf production.

There were two differences in results between the 2010 and 2011 experiments testing allocation response to manual larval and adult weevil damage: apical damage increased clonal offspring production only in the 2010 experiment, while axillary damage increased leaf production only in the 2011 experiment. These differences in plant responses may be due to differences in the duration of the experiments and timing of data collection. The 2010 experiment surveyed for responses after 37 days, and perhaps this longer time period allowed clonal responses to apical damage to manifest. In contrast, although the 2011 experiment surveyed for responses after 11 days, the axillary damage treatment was ongoing over those 11 days, and this higher level of damage may have been necessary to elicit a response to damage. Together, changes in leaf and clonal offspring production following damage support trade-offs between sexual and asexual reproduction, but also indicate that different responses to damage occur on different time scales or after different levels of damage. Trade-offs have be difficult to quantify in many plants, but when measured in terms of meristems that can produce either a sexual or asexual structure, as in *Polygonom arenastrum* (Geber, 1990), *Opuntia engelmannii* (Bowers, 1996), or *E. crassipes*, they may be more evident.

Pollination treatments did not influence subsequent flower or clonal offspring production, indicating that successful pollination neither elicits an allocation shift away from growth or asexual reproduction to produce additional flowers, nor does it shift resources away from flowering, as has been found in some systems (Harder and Johnson, 2005). A lack of priority given to sexual reproduction does not appear to hinder population growth in *E. crassipes* invasive populations, which grow primarily through asexual means (Bock, 1969). *Eichhornia crassipes'* continued investment in numerous showy flowers makes little sense in light of the lack of pollinators in the invaded range. Results from the common garden studies (discussed below), however, suggest that the three north Florida populations studied here, which are at least 20 km apart, have little genetic differentiation in growth and reproductive traits. Although I have no measure of within-population genetic variation, it may be possible that plants in these populations have little enough genetic differentiation to reduce response to selection decreasing sexual reproduction.

Indirect influence of damage via induced resistance

The manual leaf damage did not induce systemic defenses, although insect damage did (Figure 18). Given this, the allocation responses to manual damage in my experiments likely reflect only the direct influence of damage on reproductive allocation, not an indirect influence though trade-offs with defense allocation. It is interesting to note that manual leaf damage did influence whole-plant herbivore choice as measured by damaged area (Figure 18). This suggests

that herbivores were less likely to feed on a damaged plant due to the presence of manually damaged tissue itself, but not due to induced plant defenses. Some herbivorous insects do respond to the chemical composition of leaf surface wax (Bernays and Chapman, 1994); disruption of the surface wax in my study may have deterred feeding. The lack of allocation responses to insect damage demonstrated in the 2012 leaf damage experiments suggest that leaf damage, whether imposed manually or by weevils, plays no role in allocation pattern, even though insect damage did induce defenses in the 2010 experiment (Figure 18). This suggests that there is no trade-off between systemic induced defense response and reproductive allocation. Trade-offs between reproduction and defense are predicted (Stamp, 2003) but are not always found (Koricheva, 2002).

Population surveys

Results from 2010 and 2011 damage experiments indicate that plants from the CRK population added more leaves and produced more clonal offspring than plants from the WAC population, and surveys of natural populations in 2011 indicated differences in leaf size, leaf number, plant density, and clonal offspring density among populations. This suggests an effect of either local environment or genetic differences among populations.

Differences in herbivory levels in natural populations may be due to either insect abundance or plant resistance levels. The STM population had more natural damage than the CRK or WAC populations, and a higher (but not significantly) weevil density (Table 7). Because STM plants received less damage (but not significantly) in no-choice trials than the other populations (Table 7), it appears that differences in natural damage levels are more likely to be due to variation in insect abundance than in resistance levels. However, common garden experiments show that after 33 days in a common environment, population differences in damage acquired in no-choice bioassays did emerge, with the CRK population showing less resistance (approximately 50% more scars) than either STM or WAC populations.

Common garden experiments suggest that differences in size and asexual reproduction among four field populations are not strongly genetically based. Although clonal offspring production still differed among populations after 53 days in a common environment, these differences were greatly reduced (Figure 20), while differences in leaf production among populations had disappeared. These results may mean that there is little genetic differentiation among *E. crassipes* populations in north Florida for size and leaf production, but there may be some genetic variation in herbivore resistance. Genetic diversity within and among populations throughout much of *E. crassipes'* invaded range is quite low (Zhang *et al.*, 2010), and the north Florida populations in this study rarely produce fruit (pers. obs.), suggesting that much of the variation in allocation to sexual versus asexual reproduction in the N. Florida range is due to plastic responses to the environment.

CONCLUSIONS

Overall, results from these experiments suggest that environments with high weevil abundance are likely to elicit increased asexual reproduction in *E. crassipes*. This pattern of asexual growth in a high-damage environment may contribute to *E. crassipes'* success despite a paucity of pollinators in its invaded range. It does not, however, explain the apparent success of biocontrol efforts using *Neochetina* species (Center *et al.*, 1999a; Center *et al.*, 1999b), since it seems to indicate that biocontrol will increase population growth through asexual reproduction. My results may conflict with previous work due to differences in damage levels imposed or

effects of natural herbivory that my study was unable to measure. Although the manual adult weevil damage imposed in these experiments was more severe than that found in the field, it may be that larval damage in the field is more severe than in these experiments, and capable of reducing *E. crassipes* population growth. It is also possible that larval damage could facilitate secondary infection by pathogens (Thaler *et al.*, 1999; but see Thaler *et al.*, 2004), which was not a factor in my experiments. While the potential for other effects of damage, such as secondary infections, warrants attention, the results described here highlight our general lack of knowledge about the effects of herbivores on asexual reproduction, and given the number of asexually reproducing plant species, asexual plant responses to the biotic environment deserve further research.

CHAPTER FIVE

CONCLUSION

The variation in plant traits seen in nature is shaped in part by the biotic environment plants experience, and plants themselves play a role in shaping that environment. We know that herbivores and pollinators can together influence host plant traits in ways that cannot be predicted when considering each group individually (Strauss *et al.*, 1999; Mothershead and Marquis, 2000; Miller *et al.*, 2008). However, there are several aspects of how herbivores and pollinators influence host plants that deserve more research, and the goals of my dissertation were to explore some of these aspects. In general, my dissertation seeks to improve our understanding of how insect herbivores and pollinators interact with plants to create variation in plant traits, and how plant traits mediate interactions between herbivores and pollinators.

My results show that herbivores and pollinators potentially limit plant size and flowering trait expression in *Chamerion angustifolium* through both within- and across-year effects. Reduction in pollen receipt and insect damage (through stigma excision and insecticide) both affected plant traits. However, while fruit set responses suggest that pollinators were responsive to both insect damage (manipulated by insecticide) and plant size and flowering traits (manipulated by stem and flower removal), insect herbivores responded only to pollen receipt (stigma excision) and were unexpectedly unresponsive to other plant traits. My results suggest that the overall effects of herbivores and pollinators on plant size and flowering traits favor intermediate trait expression.

I found indirect effects in both directions between herbivores and pollinators; while indirect effects of herbivores on pollinators are somewhat common (Lehtilä and Strauss, 1997; Steets and Ashman, 2004), effects in the other direction are rarely demonstrated. However, failure to demonstrate pollinator effects on leaf herbivores may be due to a failure to look for them, and my results suggest they should be considered.

The across-year effects of both herbivores and pollinators in this study emphasize the need for multi-year studies on perennial plants. Across-year effects of herbivores and pollinators have been found in other systems (e.g., Snow and Whigham, 1989; Sacchi and Connor, 1999; Pratt *et al.*, 2005; Sletvold and Ågren, 2011), but single-year studies of perennial plants are still common.

When multi-year studies are not possible, modeling can provide predictions of lifetime effects. My model agrees with predictions for evolution of allocation schedule in non-variable environments (Cohen, 1971; Perrin and Sibly, 1993), and predicts selection for late flowering in annuals and for simultaneous growth and flowering in perennials in variable herbivory and pollination environments. While other models do not predict evolution of late flowering for annual plants, the inclusion of both antagonism (herbivory) and mutualism (pollination) may favor a late-flowering phenotype that takes advantage of occasional late peaks in pollination. In this case, correlated variability in herbivory and pollination, which may be likely for insect foragers responding similarly to abiotic conditions, may have led to this unexpected outcome.

In both previous chapters, I focused on plant size and flowering traits, but the prevalence of clonal plants in nature, as well as the importance of clonal reproduction in influencing plant population characteristics, makes understanding clonal responses to herbivory and pollination important. My results from chapter four indicate that asexual reproduction in *Eichhornia crassipes* is indeed an important consideration when measuring effects of insect foragers: asexual

reproduction as well as individual plant growth increased in response to larval-type damage. This suggests that herbivores can have important consequences for management of clonal invasive plants, if use of herbivores of biocontrol unexpectedly increases population growth. It also suggests that we should not assume that both herbivores and pollinators will be equally important for flowering plants, since *E. crassipes* produces many showy flowers but does not seem to invest in seed production at the expense of either growth or asexual reproduction. This research adds to the scarce knowledge that we have about clonal plant responses to herbivory, which suggests that asexual reproduction is favored (Meyer and Root, 1993; Bråthen and Junttila, 2006; Wise *et al.*, 2006), clonal plant responses to pollination, for which we have to my knowledge no information.

My dissertation proposed to inform our understanding of how insect herbivores and pollinators interact with plants to create variation in plant traits, and how plant traits mediate interactions between herbivores and pollinators. My results demonstrate (1) the importance of considering multiple forager types to understand the expression of plant traits, (2) that plant traits may reflect past as well as present environmental conditions, so across-year effects should not be overlooked, (3) that variability in herbivory and pollination together might shape the evolution of plant traits in unexpected ways; and (4) that measuring plant asexual reproduction is vital to our understanding of plant responses to foragers.

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BIOGRAPHICAL SKETCH

A third-generation Oregonian, Amanda attended the University of Oregon and is proud to be a Duck, but some of her best friends are Beavers. She graduated with a Bachelors of Arts in Spanish and Exercise & Movement Science in 2001. She joined the Ecology and Evolution Group at the Florida State University in 2005 to study plant-insect interactions with Nora Underwood and Brian Inouye.

Degrees

PhD. Biology, Florida State University, 2012. Plant responses to joint effects of herbivores and pollinators. Supervisors: Nora Underwood and Brian Inouye.

BA. Spanish and Exercise & Movement Science, University of Oregon, 2001.

Awards/Honors

Robert K. Godfrey Endowment for Botany, Florida State University, 2011
Julia Morton Invasive Plant Research Grant, Florida Exotic Pest Plant Council, 2011
Dissertation Research Grant, Florida State University, 2009
University Fellowship, Florida State University, 2009, 2007, 2005
NSF Graduate Research Fellowship, Honorable Mention, 2007
Robert B. Short Scholarship in Zoology, Florida State University, 2006
Departmental Honors (Spanish), University of Oregon, 2001
Centurion Award, University of Oregon, 2001
Phi Beta Kappa Honor Society, 2000
International Education and Exchange Scholarship, University of Oregon, 2000
Dean's List, University of Oregon, 1996-2001
Presidential Scholarship, University of Oregon, 1996-2000

Publications

Holland, J.N., A. Buchanan, and R. Loubeau, 2004. Oviposition choice and larval survival in an obligately pollinating granivorous moth, Evolutionary Ecology Research, 6: 607-618.

Presentations

Attracting pollinators and avoiding herbivores: resource allocation in a perennial plant". Ecological Society of America Annual Meeting, Austin, TX, 2011

Insect foragers and plant allocation pattern: within- and among-year effects in *Chamerion angustifolium*. Ecology and Evolution across Trophic Levels, Bucknell University, Lewisburg, PA, 2011

Insect foragers and plant allocation pattern, Ecology and Evolution Seminar, Florida State University, Tallahassee, FL, 2011

A dynamic optimization model of the influence of allocation pattern and herbivore and pollinator environments on plant fitness, Ecological Society of America Annual Meeting, Albuquerque, NM, 2009

Evolutionary consequences of plant phenology, herbivory, and pollination, Southeastern Ecology and Evolution Conference, Gainesville, FL, 2009

Professional appointments and development

Curriculum Development: Chemical Ecology, A. Winn, Florida State University, 2010
Research assistant, B. Inouye, Florida State University, 2008
Research assistant, N. Underwood, Florida State University, 2006
Graduate, "The Bee Course", Southwestern Research Station, Portal, AZ, 2005
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Research assistant, J.N. Holland, University of Arizona, 2001

Undergraduate mentoring

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Rachel Atchison, Florida State University. Root herbivory by banded cucumber beetles (*Diabrotica balteata*) affects zucchini plant performance (*Cucurbita pepo*), 2009-2010
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Teaching

Experimental Biology (BSC3402L) teaching assistant, FSU, 2009, 2012
Guest teacher presenting "Insects as Food", School of Arts and Sciences, Tallahassee FL, 2012
Plant Biology Lab (BOT3015L) teaching assistant, FSU, 2011
Guest teacher presenting "Art and Ecology", Pineview Elem. School, Tallahassee FL, 2011
Anatomy and Physiology Lab II (BSC2086L) teaching assistant, FSU, 2011
Plant Biology (BOT3015) teaching assistant, FSU, 2010
Research Methods (ISC3523C) teaching assistant, FSU, 2010

General Ecology (PCB3043) teaching assistant, FSU, 2007, 2008
Anatomy and Physiology Lab I (BSC2085L) teaching assistant, FSU, 2006
Teacher, Biology/Integrated Science, South Ruimveldt Secondary School, Guyana (U.S. Peace Corps), 2003-2004

Service

Graduate student representative, Department Chair Search Advisory Committee, FSU, 2012
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