ABSTRACT

Title of Document: QUANTIFYING CONTEXT-DEPENDENT OUTCOMES OF THE INTERACTION BETWEEN *SILENE STELLATA* (CARYOPHYLLACEAE) AND ITS POLLINATING SEED PREDATOR, *HADENA ECTYPA* (NOCTUIDAE), A POTENTIAL MUTUALIST

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Interactions with variable outcomes are particularly useful in allowing for the exploration of ecological conditions that give rise to and allow persistence of mutualistic interactions. Understanding the context and conditions under which outcomes of mutualistic interactions vary is critical to understanding their ecology. Of insect-plant mutualisms, pollination by pollinating seed predators is a unique interaction in which flowers and fruits are food for the pollinator's young, and outcomes range from obligate (e.g., figs–fig wasps) to facultative (e.g., *Silene–Hadena*). The facultative nature of *Silene-Hadena* interactions makes them ideal for a study of the role of ecological conditions in determining interaction outcomes and consequently may inform us of the conditions promoting mutualisms. My goals were to explore variation in the interaction outcome between *Silene stellata* and its pollinating seed predator, *Hadena ectypa*, under different

ecological conditions and, in addition, to understand the role of plant traits in attracting oviposition and the role of oviposition in determining interaction outcomes. My research demonstrates that plants with longer corolla tubes had higher oviposition rates in each year, and I observed significant positive relationships between oviposition and predation and oviposition and fruit initiation. Further, this interaction is antagonistic for spatially isolated plants because low pollination levels of isolated plants resulted in lowered seed set compared with non-isolated plants, and predation was significantly higher for isolated plants. Finally, the magnitude of phenological synchrony between *S. stellata* flowering and *H. ectypa* oviposition and the effect of synchrony on flower and fruit predation varied between seasons. This interannual variability in the effect of synchrony on predation may be attributed to significant differences in within season patterns of flowering and oviposition. My research demonstrates a link between oviposition and host plant traits, the role of oviposition in host plant reproduction and the identification of two ecological scenarios under which the interaction outcomes between *S. stellata* and *H. ectypa* vary. This variation under different ecological scenarios, along with positive relationships between oviposition and both predation and fruit initiation, demonstrates a mechanism for the persistence of this interaction and other facultative pollinating seed predator interactions.

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Dedication

To RRK, VMK and BK2

Acknowledgements

I would like to begin by thanking my husband, Bob Kula, for all his support, patience and understanding. His help in the field at the end of the 2008 season made the end of that long season more bearable and provided us one last opportunity to do field work together before becoming parents. His help as chief child care provider and cook for two weeks at the end of the 2009 field season at Mountain Lake was a special time for us as a family and allowed me to (nearly) seamlessly and more fully blend family and research for the first time. That time was a unique opportunity for him to develop a closer relationship with Vincent and was invaluable on their long drive home from the station and in the following week when they were on their own at home while I finished up the field season. Furthermore, it prepared them for their next fairly intense experience together 2.5 years later as I had a last big push to finish my "big homework assignment." I know of very few husbands and fathers who could handle so easily the arrangement that was required of this exceptional situation.

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and I am a better ecologist now with that knowledge and experience. Finally, their model of balancing family and science gave me confidence to pursue my interests in science while my family grew.

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Chapter 1

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Chapter 1

Choices and consequences of oviposition by a pollinating seed predator, *Hadena ectypa* (Noctuidae), on its host plant, *Silene stellata* (Caryophyllaceae)

Abstract

Pollinating seed predators have dual effects on host plant fitness, via pollination and flower and fruit predation, and their oviposition preferences may be influenced by plant floral and size traits. If oviposition is associated with pollination and predation, conflicting selection pressures on preferred traits may occur. Oviposition may influence plant reproductive success by increasing stigmatic pollen loads and/or the quality of pollen deposited. I quantified oviposition by the pollinating seed predator, *Hadena ectypa*, on its host, *Silene stellata*, to determine if plant traits were associated with oviposition and whether oviposition was related to flower and fruit predation or fruit initiation over three years. I quantified stigmatic pollen loads of flowers visited by *H. ectypa* that nectared or both nectared and oviposited. I compared seed germination from fruits with and without an egg to determine if oviposition increased pollen quality. Plants with longer corolla tubes had higher oviposition rates. Oviposition was associated with other traits only in some years. Oviposition did not increase stigmatic pollen load or seed germination. I observed significant positive relationships between oviposition and predation and oviposition and fruit initiation. Together these results suggest that *Hadena ectypa* has the potential to exert conflicting selection pressures via its dual role as

pollinator and seed predator, and the specific traits under these potential selective pressures may vary temporally. Positive relationships between oviposition and both predation and fruit initiation suggest a mechanism for the maintenance of this facultative pollinating seed predator interaction.

Introduction

Studies of the associations between plants and their pollinators originated with Sprengel (1793) and Darwin (1862). These early observations have resulted in a large literature demonstrating pollinator preference for particular floral traits. Pollinators visit flowers based on morphological traits such as flower or petal size (Ashman and Stanton 1991, Fenster et al. 2006, Gomez et al. 2008) and corolla tube length (Galen and Cuba 2001, Gomez et al. 2008, Dudash et al. 2011) due to an association of these floral traits with greater nectar rewards (Faegri and van der Pijl 1979, Armbruster et al. 2005, Fenster et al. 2006). Indeed, the evolution of floral syndromes provides much circumstantial evidence for pollinator preference for particular floral traits (Fenster et al. 2004, Armbruster et al. 2011). Furthermore, through their visitation preferences, pollinators have been shown to play an important role as selective agents in the evolution of floral traits (Reynolds et al. 2010).

Insect herbivores, florivores and frugivores also express preference for morphological floral or plant traits, particularly through female oviposition preferences. As they are for pollination, larger flowers or petals are more attractive for oviposition (Kudoh and Whigham 1998), and females choose oviposition sites based on specific floral traits (e.g., shorter, wider corolla tubes) (Brody 1992, Zimmerman and Brody 1998, Cariveau et al.

2004). Insects prefer to oviposit on plants that are taller (Cariveau et al. 2004, Nowicki et al. 2005) or with longer stems (Craig et al. 1989, Nozawa and Ohgushi 2002) or wider stems (Agrawal and Van Zandt 2003) and those with more flowers (Holland et al. 2004, Nowicki et al. 2005). While the effects of leaf herbivory may indirectly affect plant fitness (Krupnick et al. 2000, Irwin and Brody 2011), seed, flower and fruit consumption result in direct loss of female and male reproductive output and are expected to have large effects on the evolution of floral traits due to their direct impact on plant reproductive output (Pilson 2000, Cariveau et al. 2004, Rey et al. 2006, Parachnowitsch and Caruso 2008, Kolb et al. 2007, Kolb and Ehrlen 2010).

Pollinators and herbivores may express similar preferences for floral or plant traits, possibly resulting in conflicting selection pressures. Conflicting selection pressures result when two selective agents with opposing effects on fitness (e.g., pollinators and predators) exert natural selection on the same trait value, and therefore the trait is selected for in both positive and negative directions (reviewed by Strauss and Whittall 2006). For example, Campbell et al. (2002) found that *Ipomopsis aggregata* flowers with wider corollas are preferred by pollinators, but flowers with wider corollas also experience higher seed predation rates compared to narrower flowers that are less attractive to both pollinators and seed predators. Additionally, both fruit initiation and fruit predation were lower for shorter *Primula farinosa* plants indicating that both pollinators and herbivores prefer taller plants (Ehrlen et al. 2002).

Pollinating seed predators (e.g., fig wasps, yucca moths, *Hadena* moths; a.k.a. nursery pollinators) have the opportunity to affect plant fitness positively as mutualists (because adults are pollinators) and negatively as antagonists (through larval feeding on

seeds, flowers, and/or fruits). Because pollinating seed predators potentially have both positive and negative effects on plant reproduction, studies of their interactions with host plants can offer insight into the dual roles of this unique interaction in the evolution of floral phenotypes (e.g., Godsoe et al. 2008).

In pollinating seed predator mutualisms, pollination is the primary benefit conferred to host plants, but there is variation in the role of oviposition during passive pollination, as compared to interactions where pollen is actively deposited at the time of oviposition, as in *Yucca*-*Yucca* moth interactions (Pellmyr 2003). For instance, oviposition is essential for pollination by *Greya* moths because they deposit pollen onto *Lithophragma* flowers only while they oviposit (Pellmyr and Thompson 1992, Thompson and Pellmyr 1992), and *Hadena compta* moths deposit four times more pollen when ovipositing versus nectaring on *Dianthus sylvestris* flowers (Collin et al. 2002). However if ovipositing moths visit female flowers preferentially (i.e. either on female plants of dioecious species or female phase flowers on plants of protandrous species, Westerbergh 2004), they are less likely to carry pollen which may result in lower stigmatic pollen loads. Additionally, if oviposition does not occur on every visit and if pollen is placed on different parts of the pollinator's body (e.g., front half of the body during nectaring versus back half of the body during ovipositing), then greater pollen carryover may result following pollination during oviposition. Greater pollen carryover may in turn increase the probability of mating between less closely related individuals resulting in higher progeny quality (Fenster 1991a, b).

The interactions between *Silene* spp. (and closely related species) and their pollinating seed predators (*Hadena* spp.) provide an ideal system to study the role of

floral and plant traits on the oviposition decisions of pollinating seed predators and the role of oviposition in pollination and reproduction (Kephart et al. 2006, Bernasconi et al. 2009). For oviposition, *Hadena* spp. have been shown to choose flowers based on number of petals and presence of the ligula at the base of the petal (i.e. coronal scale) (Brantjes 1976b) and overall flower size (Collin et al. 2002). Through seed predation, *Hadena* spp. may exert selection on flower size, size dimorphism between sexes and flower timing (Biere and Honders 1996, Biere and Honders 2006, Collin and Shykoff 2010). *Silene latifolia* plants in North America, where they are not native and have escaped their *Hadena* seed predators, have evolved to invest more in reproduction and growth and less in defense, further suggesting a role for *Hadena* as an important selective agent on the evolution of *S. latifolia* life history traits (Blair and Wolfe 2004).

Here I studied oviposition choice by *Hadena ectypa* for multiple traits of *Silene stellata* and the consequences of *H. ectypa* oviposition for several components of *S. stellata* reproduction. I measured plant floral and size traits for over 100 plants for three years and counted the number of *S. stellata* neighbors for two years. At the time of floral measurement, I quantified oviposition to determine whether *H. ectypa* showed oviposition preference for plants exhibiting particular traits or number of neighbors. I quantified stigmatic pollen loads from visits when *H. ectypa* nectared only versus nectared and oviposited. I also calculated seed germination rates from fruits with and without an egg. Finally, I determined the proportion of flowers to initiate fruit and proportion of flowers and fruits eaten by *H. ectypa* larvae to further quantify the consequences of oviposition for host plant reproduction. I asked: 1) When making oviposition decisions, does *H. ectypa* discriminate based on plant floral or size trait

expression or, additionally, surrounding plant density? 2) Does oviposition increase stigmatic pollen loads or result in greater seed germination success? And 3) Is oviposition related to initiated fruit set (an indication of pollination) or flower and fruit predation of host plants?

Methods

Study organisms—*Silene stellata* is a native, long-lived, herbaceous perennial plant that grows in forests or open meadows across the eastern half of North America. The white, fringed flowers are hermaphroditic and protandrous and exhibit a nocturnal moth pollination syndrome: anthers dehisce and stigmas become receptive in the evening, and nectar and scent production also are highest at dusk (Reynolds et al. 2009). Flowers have a mean of 25 ovules each (Reynolds et al. 2009). An important pollinator is the specialist seed predator moth, *Hadena ectypa* (Reynolds et al. 2012). Adults of this moth feed on *S. stellata* nectar and then, during approximately 40% of all *H. ectypa* visits or nearly all female *H. ectypa* visits (Kula et al., Unpublished data), female moths oviposit inside the calyx on or near the base of the ovary and occasionally on the inside surface of the calyx. *Hadena ectypa* larvae feed on *S. stellata* flowers and fruits, often consuming the entire unhardened ovary wall of flowers and all seeds within fruits. Larvae may consume up to 40 flowers or fruits to complete development under laboratory conditions (Reynolds et al. 2012). Other nocturnal moth co-pollinators that do not lay eggs on *S. stellata* are equally effective pollinators (Reynolds et al. 2012), but they do not become a prominent component of the pollinator community until the latter half of the flowering season after flowering density has peaked (Reynolds et al. 2012, Chapter 3).

Study site—My research was conducted at a clear cut meadow near Mountain Lake Biological Station in the Allegheny Mountains of southwest Virginia, U.S.A. (37°20’53”N, 80°32’41”W, elevation ≈ 1,100–1,300 meters). During 2003 and 2005, 142 plants (71 per year) were haphazardly chosen and flagged at 10 m intervals up the mountainside in three parallel transects 10 m apart. Most of the plants in these transects were included in this study (the number of plants measured each year is listed below). Wire cages with 5 cm openings were placed over all individual plants to prevent deer browsing but allow free movement of pollinators and insect herbivores. I chose to exclude deer browsing because of my explicit interest in *H. ectypa* oviposition and its role on floral and plant traits. The flowering season at this site is July through September.

Data collected—To determine which, if any, plant floral traits serve as cues for oviposition choice by *H. ectypa*, plants were examined for female phase flowers on most mornings during the flowering season each year (2007: N=120 plants; 2008: N=126; 2009: N=121). Stigmas are receptive for one to three days, and to ensure consistency, only flowers that first became female during the previous night (hereafter “day 1 females”) were selected for floral measurement and assessed for the presence of an egg. Floral measurements were taken on approximately five flowers per plant (total flowers measured, mean number measured per plant: 2007: N=536, 4.5; 2008: N=581, 4.7; 2009: N=559, 4.6). The following traits were measured to 0.1 mm with dial calipers and were natural log transformed: length of the corolla tube, width of the corolla tube opening, length of the largest petal, width of the largest petal and distance from the nectary at the

base of the flower to the tip of the stigma (hereafter “nectar-stigma distance”) (Fig. 1). Stigma exertion was calculated from nectar-stigma distance minus the length of the corolla tube. I also quantified petal dissection (hereafter “number of lobes”) (Fig. 1). These traits were chosen because they are frequently associated with different pollination syndromes and hence are associated with pollinator attraction and efficiency of pollen transfer (Fenster et al. 2004). Means of floral trait measurements from all measured flowers on each plant were calculated to obtain one representative floral trait measurement for each trait per plant in each year. The number of measured flowers with an egg was counted at the time of measurement in the field by using a hand lens with 15x magnification.

Plant size measurements were taken once for each plant each year. The number of stems was counted and plant height (cm) was measured from the base of each plant at ground level to the top of the longest stem (natural log transformed). In 2008 and 2009, the number of neighboring *S. stellata* plants within a 1 m radius around each target plant was counted to estimate local host plant density within this large continuous population of *S. stellata*.

Near the end of the flowering season, all flowers were collected from study plants after fruit maturation but before fruit dehiscence (approximately three weeks after flower opening). In the laboratory, for each plant I determined the total number of flowers (an indication of plant size), number of fruits initiated, and number of flowers and fruits consumed by larvae.

I used two metrics to assess the positive and negative outcomes of the pollinating seed predator interactions. Proportion of flowers to form fruits (i.e. proportion of flowers

pollinated) was calculated as the number of fruits (both those with seeds and those with seeds eaten) divided by total number of flowers produced on a plant, and this metric is hereafter referred to as "initiated fruit set." Proportion of flowers and fruits that were eaten by *H. ectypa* larvae was calculated as number of flowers and fruits consumed divided by total flower production on each plant and is hereafter referred to as "predation." Because they were calculated as proportions, initiated fruit set and predation were arcsine square-root transformed prior to analyses.

In 2008, I quantified stigmatic pollen loads following single pollinator visits where *H. ectypa* either nectared only or both nectared and oviposited. Groups of 5–12 potted plants grown from seeds collected from the study site in 2006 and 2007 were placed within my field site and observed on 11 evenings from 11–25 July. After a visit by *H. ectypa* to a day 1 female flower, the type of visit was noted (i.e. nectaring only versus nectaring followed by ovipositing), and the flower was collected and placed individually in a glass vial. Within 90 minutes, flowers were transported to the lab where stigmas were removed then fixed and stained in fuchsin-containing glycerin jelly on a microscope slide (Beattie 1981). Pollen grains deposited on stigmas were counted using a compound microscope slide at 40x magnification. For analysis, total stigmatic pollen loads per flower were square-root transformed.

While processing flower and fruits from all three years, I set aside samples from plants that I knew from field data had at least one flower with an egg and one flower without an egg. Laboratory assessment confirmed whether the flower subsequently formed a fruit and/or was eaten by larvae. From these plants, the uneaten fruits with and without an egg were used to examine the effects of oviposition on probability of seed

germination. Seeds were counted then placed on moist soil in 5 cm pots during December of the year following fruit collection and cold treated outdoors for 2.5 months. In mid-March, pots were moved indoors and checked for seedlings every 4–5 days. Seed germination rate was calculated as the number of seedlings that emerged out of the number of seeds sown for each fruit, and for analysis, the proportion was arcsine square-root transformed.

Statistical Analyses—Because there was consistent high correlation between petal length and petal width ($P<0.0001$; 2007: r=0.35, 2008: r=0.53, 2009: r=0.55), a new composite trait, petal area, was calculated by multiplying petal length and width which captures most of the area of the petal (as for *Silene virginica* in Fenster et al. 2006). Petal area was then square-root transformed to maintain dimensionality among traits. Likewise, stem number was consistently correlated with total flower production per plant ($P<0.0001$; 2007: r=0.55, 2008: r=0.74, 2009: r=0.51) and was not included in further analyses.

To obtain an overall measure of plant floral size, I performed principal components analyses for each year on corolla tube length and width, square-root transformed petal area, stigma exertion and number of petal lobes. I used the first principal component (hereafter referred to as "PC1") from each year to reduce the dimensionality among these variables and to represent general floral size.

I modeled oviposition (the number of flowers with an egg out of the total number of flowers sampled) as a linear function of plant floral and size traits and number of neighbors, as well as initiated fruit set and predation, to capture the pattern of moth

oviposition preference for these traits and the outcome of oviposition for the host plant over three years. I employed generalized linear models, which use maximum likelihood to estimate model parameters, and I specified a binomial probability distribution (number of flowers with an egg out of total number of flowers) with a logistic link function in Proc Genmod (SAS version 9.2). Plant floral trait models for each year included corolla tube length, corolla tube width, petal area, stigma exertion and number of petal lobes; analyses were performed on transformed measurements, as noted above. General flower size (PC1) was modeled separately. The plant size models included natural log transformed plant height and total flower production per plant annually. For 2008 and 2009, the effect of neighboring *S. stellata* plant density was also examined.

I used a mixed model ANOVA (Proc Mixed, SAS, version 9.2) to test for differences in stigmatic pollen loads onto stigmas during single visits when *H. ectypa* either nectared versus both nectared and oviposited. Random factors included the plant number from which the sample was taken and the proportion of male flowers in the patch on the night that a sample was collected.

I also used a mixed model ANOVA (Proc Mixed, SAS, version 9.2) to test for differences in seed germination from fruits with and without an egg. Plant number was included as a random factor to take into account that at least two fruits (with and without an egg) from each plant were included in the experiment.

Results

Each plant floral and size trait ($P<0.001$), in addition to proportion initiated fruit ($P=0.003$) and predation ($P<0.0001$), significantly differed among years. Therefore data

from the three years of study were analyzed separately. Analyzing years separately further eliminated possible complications with repeated measurements on plants for multiple years.

The mean ± 1 standard error oviposition rate per observed flowers was 0.17 ± 0.02, 0.22 ± 0.07 and 0.30 ± 0.03 in 2007, 2008 and 2009, respectively.

Of the plant floral traits measured, only corolla tube length was significantly related to oviposition rate in all three years: plants with flowers exhibiting longer corolla tubes had higher probability of oviposition (Tables 1, 2). In 2009 only, petal area also was positively associated with the probability of a flower receiving an egg (Tables 1, 2).

The first principal component of overall plant floral size was related to oviposition only in 2009 (Tables 1, 2). The relationship was positive: plants with larger flowers received more eggs per flower. All five floral traits were significantly positively loaded on PC1 ($P<0.0001$), and PC1 explained 37, 33 and 35 percent of the total variance in floral traits in 2007, 2008 and 2009, respectively.

For plant size traits, the results varied by year. Total flower production per plant demonstrated a significant negative association with probability of oviposition in both 2007 and 2009: plants with fewer flowers had a higher probability of oviposition (Tables 1, 2). In 2009 only, plant height was also positively associated with oviposition within a flower. Number of neighboring *S. stellata* plants was not significantly associated with probability of oviposition in either 2008 or 2009.

Stigmatic pollen loads did not differ between flowers visited for nectaring only versus both nectaring and ovipositing ($F_{1,50}=0.07$, $P=0.7915$, Table 3).

In all three years, seed germination did not differ when a egg was present or absent

within a flower (2007: $F_{1,56}$=1.01, *P*=0.3204; 2008: $F_{1,90}$=0.02, *P*=0.8807; 2009: $F_{1,25}$=0.21, *P*=0.6474; Table 4).

Predation of flowers and fruits was significantly positively associated with oviposition in all three years (Table 1, 2). The association between initiated fruit set and egg deposition per flower was not significant at *P*<0.05 for any given year (Tables 1, 2). However, because the sign of the relationship between initiated fruit and oviposition was always positive and *P* values were always <0.2, I estimated a combined *P* for all three years together to further examine this relationship (Sokal and Rohlf 1995). I first took the quantity -2∑ln *P* across the three years. The degrees of freedom were 2*3 years = 6. Because -2∑ln *P*=13.18 is greater than $\chi^2_{0.05,6}$=12.59, I rejected the null hypothesis that the relationship is independent among years. Thus I detected a similar and significant positive signal across all years overall between initiated fruit set and egg deposition.

Discussion

The pollinating seed predator *Hadena ectypa* demonstrated oviposition preference for plant floral and size trait expression. In particular, *H. ectypa* oviposition was higher on *S. stellata* plants exhibiting longer corolla tubes in all three years. However, number of neighboring plants did not affect oviposition. Oviposition did not increase stigmatic pollen loads or seed germination rate from fruits with an egg. Predation of flowers and fruits was significantly positively related to oviposition in all three years, and the overall relationship between initiated fruit set and oviposition was positive and significant across years. Through positive (via pollination and subsequent fruit initiation) and negative (via oviposition and subsequent flower and fruit predation) effects on plant reproduction, *H.*

ectypa oviposition preference for particular plant floral and size traits demonstrates its potential to act as an agent of selection on *S. stellata* at both the larval and adult stages.

In an optimal foraging theoretical framework (Pyke et al. 1977), pollinating seed predators choose to visit flowers based on their nutritional needs, in this case, nectar. Corolla tube length and petal area (or flower size) advertise pollinator rewards, especially nectar, in a diversity of plant species (Ashman and Stanton 1991, Creswell and Galen 1991, Gomez et al. 2008), including *Silene virginica*, a close relative of *S. stellata* (Fenster et al. 2006). Because of the very low volume of nectar typically found in *S. stellata* flowers (Reynolds et al. 2009), I was not able to test for a correlation between plant floral trait expression and nectar quantity or quality. However, female *H. ectypa* moths feed on nectar before ovipositing (personal observation), indicating a behavioral link between nectaring and ovipositing behaviors, and there is some evidence that nectar plays a role in oviposition decisions of other pollinating seed predators and herbivores (Brantjes 1976a, Thompson and Pellmyr 1992, Pellmyr and Thompson 1992, Adler and Bronstein 2004). Aside from providing a basic nutritive reward for pollinators, nectar also can provide specific oviposition stimulants (Wehling and Thompson 1997) and other essential nutrients (e.g., amino acids, Erhardt and Rusterholz 1998).

Alternatively, optimal oviposition theory predicts that oviposition sites should be chosen to optimize larval growth and survival because females have increased fitness through the success of their offspring on oviposition substrates that provide adequate resources or protection (Jaenike 1978, Thompson 1988). For instance, females lay eggs in flowers that are more likely to produce seeds (Brody and Morita 2000), and yucca moths actively pollinate at the time of oviposition to ensure larvae have ample seed resources

(Pellmyr et al. 1996). Because *H. ectypa* larvae require multiple fruits to complete their development before pupation (Reynolds et al. 2012), the number of flowers on a plant could indicate an increased concentration of rewards for moth larvae, and other studies have found a positive relationship between number of flowers and oviposition (Holland et al. 2004, Nowicki et al. 2005). However, the significant relationship I found between oviposition and total flower production in two years was negative: plants with fewer flowers had a higher probability of receiving an egg laid within a given flower. This result was not simply an issue of my assessing oviposition in a smaller proportion of total flowers on larger plants (an average of 4–5 flowers on plants were sampled on plants regardless of total number of flowers). In a separate study in 2009, I assessed oviposition on a mean of 90% of flowers on 97 plants, and the relationship of oviposition rate and total flowers for those plants is also significant and negative (Kula et al., Unpublished data). Furthermore, total flower production per plant was not correlated with any plant floral or size traits that were significantly related to oviposition (e.g., corolla tube length) in any of the three years of study ($P>0.1$), except for plant height in 2009 ($P<0.0001$). A negative correlation might arise between oviposition and total flower production if female moths spend a similar amount of time per plant laying eggs on each plant they visit and lay only one egg per plant. My observations confirm that *H. ectypa* females typically oviposit only one egg per visit to a plant, independent of plant size. It is also possible that number of flowers on a plant is affected by another factor leading to indirect effects on oviposition rates (e.g., larval parasitism, Price et al. 1980).

An additional possible benefit for oviposition into flowers with longer corolla tubes may be protection for larvae. Egg mortality from desiccation due to exposure can be high

(Zimmerman and Brody 1998), and deeper corolla tubes may provide a more humid environment, which may increase larval survival (Wilson et al. 1982). In addition, parasitoids likely pose a potential major threat to *Hadena* larval survival (Elzinga et al. 2003), and larvae from eggs laid where exposure to parasitoid attack is minimized may have higher survival (Chen and Welter 2007).

Unlike *Greya* moths which deposit much more pollen on stigmas of *Lithophragma* spp. during oviposition visits (vs. nectaring visits) (Pellmyr and Thompson 1992, Thompson and Pellmyr 1992), *H. ectypa* was similarly efficient at pollination of *S. stellata* during nectaring visits and visits that involved both nectaring and ovipositing. These similar pollination levels demonstrate that increased time spent on and activity at *S. stellata* flowers during *H. ectypa* oviposition does not increase pollen transfer. The similar germination rate of seeds from fruits that did or did not receive an egg was reflective of similar stigmatic pollen loads from the two types of visits and may additionally indicate that pollen transferred during oviposition does not reflect greater carryover distance. Therefore, oviposition and initiated fruit set are positively related only because pollination by *H. ectypa* occurs during visits where moths oviposit. This result follows that of Reynolds et al. (2012) who showed that oviposition rate on haphazardly collected flowers in the morning is a good proxy for pollinator visitation rates on the previous night.

Because of a positive association of oviposition with both fruit initiation and flower and fruit predation together with a consistent positive association between oviposition and corolla tube length, *H. ectypa* moths may exert conflicting selection pressures on corolla tube length. The total potential effect of *H. ectypa* as a selective agent on floral

design is likely influenced by inter-annual and geographic variation in its roles as both an important pollinator and flower and fruit predator of *S. stellata* (Reynolds et al. 2012). In years when or sites where *H. ectypa* is a more important component of the pollinator community, there may be stronger positive selection for plants with longer corolla tubed flowers because those flowers will be pollinated when females visit to oviposit. Alternatively, when or where predation by *H. ectypa* outweighs its contributions to pollination, there will be stronger negative selection on plants with longer corolla tubes. Indeed, in a study of natural selection on floral traits, Reynolds (2008) showed that for *S. stellata,* corolla tube length was under negative directional selection but only in years with the highest *H. ectypa* predation rates. These results are consistent with prior literature demonstrating that the combined effects of pollinators and seed predators may result in year-to-year variation in the magnitude and direction of phenotypic selection on corolla tube length (Campbell 1991, Zimmerman and Brody 1998). Variation in the outcome of interactions between host plants and pollinating seed predators may also explain inter-annual variation in the significance of an association between *H. ectypa* oviposition and some *S. stellata* traits (i.e. petal area, flower size, plant height and flower production).

Finally, the positive relationships between oviposition and both fruit initiation and flower and fruit predation indicate a key mechanism for the maintenance of this (and other facultative) pollinating seed predator interaction and may possibly explain why no avoidance mechanisms by *S. stellata* to its pollinating seed predator have evolved. *Silene-Hadena* interactions are hypothesized to be some of the least specialized of pollinating seed predator interactions, and it is these less specialized interactions that will potentially

explain the pathway toward mutualism for host plant–pollinating seed predator interactions (Dufay and Anstett 2003).

Tables

Table 1. Untransformed means ± 1 standard error of plant floral and size traits, number of neighbors, initiated fruit set and predation by *Hadena ectypa* of *Silene stellata* across three years from the study population near Mountain Lake Biological Station, Pembroke, VA.

	2007 N=120 Mean ± S.E.	2008 N=126 Mean ± S.E.	2009 N=121 Mean ± S.E.
Flowers measured per plant	4.47 ± 0.11	4.61 ± 0.09	4.62 ± 0.08
Floral Traits			
Corolla tube length	10.36 ± 0.08	10.48 ± 0.09	9.95 ± 0.08
Corolla tube width	8.19 ± 0.09	7.46 ± 0.10	7.69 ± 0.10
Petal area	108.25 ± 1.71	99.89 ± 1.78	114.93 ± 1.75
Stigma exertion	9.60 ± 0.12	7.94 ± 0.15	8.87 ± 0.12
Petal lobes	12.30 ± 0.26	10.94 ± 0.20	11.29 ± 0.23
Size Traits			
Height	86.34 ± 1.67	77.49 ± 1.52	79.08 ± 1.64
Total flowers	70.81 ± 7.78	100.07 ± 11.0	53.53 ± 6.51
Neighbors	--	4.80 ± 0.31	2.78 ± 0.17
Initiated fruit set	0.55 ± 0.02	0.65 ± 0.02	0.60 ± 0.02
Predation	0.55 ± 0.03	0.37 ± 0.02	0.52 ± 0.02

Table 2. Results from generalized linear models of the relationships of *Silene stellata* plant floral and size traits, number of neighbors, initiated fruit set and predation by *Hadena ectypa* with oviposition by *H. ectypa*. Traits in bold have a significant relationship with oviposition.

	2007			2008			2009		
Source	Sign	χ^2	*P*	Sign	χ^2	*P*	Sign	χ^2	*P*
Floral Traits									
Corolla tube length	**+**	**6.88**	**0.0087**	**+**	**8.45**	**0.0036**	**+**	**4.47**	**0.0344**
Corolla tube width		2.5	0.1139		0.31	0.5783		0.13	0.7173
Petal area		0.3	0.5668		0.84	0.3596	**+**	**5.93**	**0.0149**
Stigma exertion		0.54	0.4614		0.25	0.6178		0.27	0.6003
Petal lobes		0.26	0.607		2.57	0.1092		0.34	0.5616
Floral Size (PC1)		0.58	0.4458		0.61	0.4364	**+**	**26.52**	**<0.0001**
Size Traits									
Height		2.1	0.1471		0.29	0.5903	**+**	**9.9**	**0.0017**
Total flowers	**-**	**8.89**	**0.0029**		0.01	0.9365	**-**	**4.73**	**0.0296**
Neighbors					0.95	0.33		2.38	0.1233
Initiated fruit set	+	2.69	0.1009	+	3.27	0.0707	+	2.16	0.1414
Predation	**+**	**7.05**	**0.0079**	**+**	**7.60**	**0.0058**	**+**	**4.47**	**0.0344**

Table 3. Untransformed means ± 1 standard error of stigmatic pollen loads of *Silene stellata* flowers that were visited once by *Hadena ectypa* moths that nectared only versus nectared and oviposited.

	N	Mean ± S.E.
Nectaring only	49	51.84 ± 6.67
Nectaring and ovipositing	20	53.40 ± 10.6

Table 4. Untransformed means ± 1 standard error of seed germination rates of *Silene stellata* from fruits that did or did not receive at least one egg from *Hadena ectypa*.

	2007		2008		2009	
	N	Mean ± S.E.	N	Mean ± S.E.	N	Mean ± S.E.
Without an egg	51	0.13 ± 0.03	75	0.32 ± 0.04	24	0.18 ± 0.05
With at least one egg	39	0.18 ± 0.05	54	0.30 ± 0.04	20	0.15 ± 0.04

Figures

Figure 1. Diagram of *Silene stellata* floral traits measured in the field. A) PW=petal width, PL=petal length, TW=corolla tube width. B) TL=corolla tube length, NS=nectar-stigma distance. Scale bars=5 mm.

Figure 2. Photograph of three *Hadena ectypa* eggs at the base of a *Silene stellata* flower with the calyx removed. Female *H. ectypa* adults insert their ovipositors into the corolla tube to deposit eggs at the base of the ovary, on the nectary or on the inside surface of the calyx. It is unusual to find more than one egg on a flower. Scale bar=1 mm.

Figure 1.

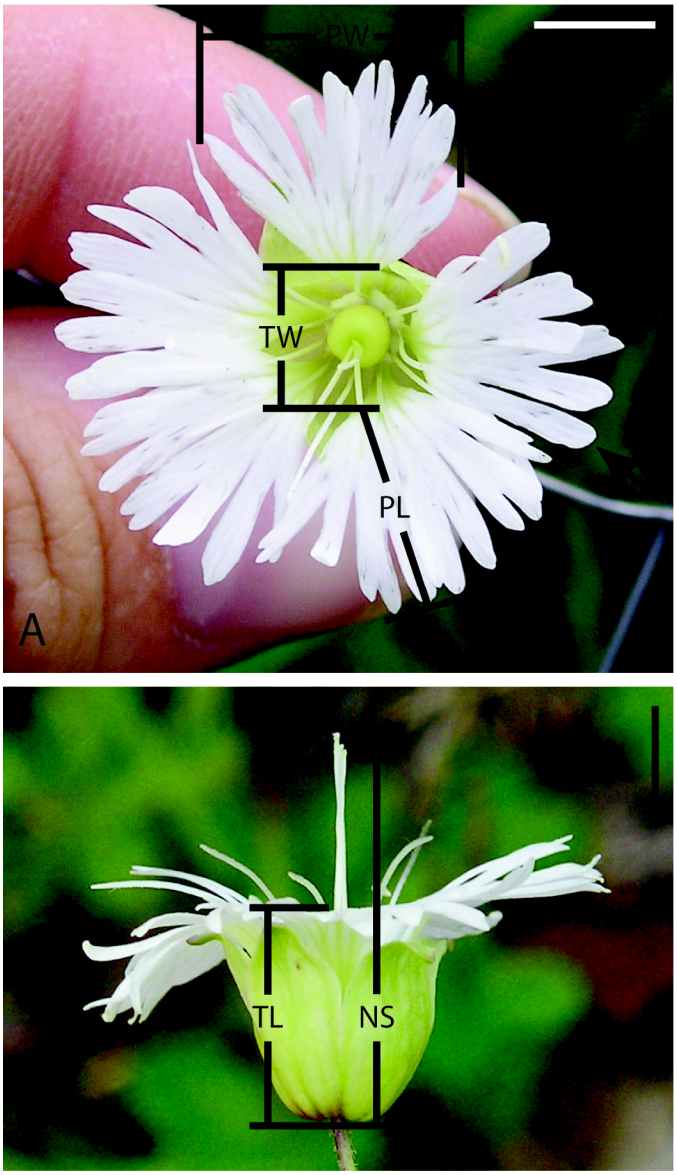


Figure 2.

Chapter 2

Isolated *Silene stellata* host plants experience decreased benefit and increased cost of interacting with their specialist pollinating seed predator, *Hadena ectypa*

Abstract

For continued persistence, small, isolated patches of plants need to successfully reproduce. Interspecific interactions often are important for host plant reproduction, but the outcome of these interactions may vary when host plants are isolated or in small patches. Pollinating seed predators have positive and negative effects on host plant reproduction, and the interaction outcome is predicted to vary under this ecological scenario. I studied the interaction between *Silene stellata*, an herbaceous perennial, and *Hadena ectypa*, its obligate pollinating seed predator. *Silene stellata* is only facultatively dependent upon *H. ectypa* for pollination because other nocturnal moth co-pollinators are equally effective at pollen transfer. Because *H. ectypa* is a specialist, dependent on *S. stellata* as a host for its progeny, I hypothesized that, for experimentally isolated plants, *H. ectypa* will become a more important pollinator (increased benefit), while predation will likely remain the same (similar cost) relative to experimentally non-isolated plants. *Hadena ectypa* was consistently more important than moth co-pollinators for both non-isolated and isolated plants. However, *H. ectypa* pollinator importance decreased for isolated plants due to lower pollen deposition in isolation compared to non-isolated plants. Seed set of isolated plants was much lower than for non-isolated plants. Oviposition was similar between the areas, but isolated plants experienced higher predation than non-isolated plants. In contrast to my initial prediction, the relative

pollinator importance of pollinating seed predators is reduced for isolated plants, resulting in decreased benefit for the host plant, and because of increased predation rates, there is an increase in cost of the interaction for the host plant. Allee effects caused by lower pollination levels and higher predation levels may limit plant population expansion or persistence in isolated patches, thereby demonstrating the importance of species interactions in conservation, especially in areas with increased habitat fragmentation.

Introduction

Small, isolated patches of plants frequently occur in nature (e.g., during colonization or after plant population fragmentation via habitat destruction), and the persistence of these patches is dependent on the successful reproduction of resident plants (Saunders et al. 1991, Debinski and Holt 2000). The outcomes of interspecific interactions that affect plant reproduction may be altered in the ecological context of isolation or low density (Root 1973, Kunin 1997, Kearns et al. 1998, Aguilar et al. 2006). For example, plants in small patches or in isolation often experience low pollinator visitation and pollen deposition rates that could directly affect plant reproductive fitness (Cunningham 2000, Aguilar et al. 2006, Andrieu et al. 2009). Likewise, levels of herbivory may increase (Fagan et al. 2005, Gunton and Kunin 2009) or decrease (Kery et al. 2001) when host plants are isolated or at low density.

Studies that simultaneously examine pollination and herbivory of host plants in isolated and non-isolated patches show that while isolated plants regularly experience lower pollination levels resulting in lower seed set, herbivory or seed predation may be higher (Jules and Rathcke 1999, Metcalfe and Kunin 2006, Garcia and Chacoff 2007) or

lower (Groom 2001) on isolated plants. Consequently, the net outcome of beneficial and detrimental interactions on isolated host plants could result in an overall increase or decrease in plant reproduction as compared to non-isolated plants. Both decreased pollination and increased herbivory that cause lower reproduction at low densities (e.g. isolation) may result in a component Allee effect: "a positive relationship between any component of individual fitness and either numbers or density of conspecifics" (Stephens et al. 1999).

Pollinating seed predators (a.k.a. nursery pollinators) have opposing effects on plant reproduction. Adult insects provide pollination services for the host plant, but females also lay eggs on or in the flowers. The pollinator's young subsequently consume the seeds or fruits. Outcomes of these interactions range from obligate where host plants and insects are dependent upon each other (e.g., figs and fig wasps, Wiebes 1979) to facultative where host plants receive additional pollination services from co-pollinators that do not consume seeds or fruits (e.g., *Silene* and *Hadena*, Kephart et al. 2006). Facultative pollinating seed predator interactions provide an opportunity to explore the ecological conditions under which seed predator pollinators are beneficial to host plants, and these conditions may facilitate the evolution and persistence of pollinating seed predator interactions (Kephart et al. 2006, Bernasconi et al. 2009).

A key to understanding the outcome of these facultative interactions for isolated host plants is consideration of the specialization of the pollinating seed predators to their host plants. If specialist pollinators visit more frequently and deposit more conspecific pollen and less interspecific pollen as compared to generalist co-pollinators (Moeller 2005), then the pollinating seed predator interaction may have a net positive outcome on plant

reproduction since all (or most) seed set would be due to pollination by these specialist visitors and not co-pollinators. On the other hand, after locating and pollinating the isolated host plants, female pollinating seed predators may oviposit resulting in high seed predation rates (Elzinga et al. 2005).

Four other studies have reported the effects of plant population size or density on interactions with pollinating seed predators. Elzinga et al. (2005) reported increased fruit predation with decreasing population size and increasing isolation for *Silene latifolia*. Across geographic scales, however, Holland and Fleming (1999) found no significant variation in fruit set, fruit maturation and fruit predation for five populations with varying *Senita* cactus density, while, Despres et al. (2007) found significant variation in costs of interactions with pollinating seed predator flies across 26 globeflower populations that differed in ecological conditions and pollinator densities. Finally, Klank et al. (2010) found no effects of population size or flower density on globeflower fly abundance, but higher population level flower density resulted in decreased predation due to a dilution effect of the flies across globeflowers. None of these studies examined the effect of isolation or plant density in terms of its influence on the beneficial aspect (pollination) of interacting with a pollinating seed predator.

My study goes further than previous studies by examining not only seed set, oviposition and seed predation but also pollinator visitation and efficiency, which together determine pollinator importance (Reynolds and Fenster 2008). Pollinator importance is a parameter that incorporates both the effectiveness of a pollinator, in terms of transfer of pollen to conspecifics, with the relative visitation frequency of that pollinator (Stebbins 1970). Stebbins (1970) suggested that pollinator importance allows

determination of the role of pollinators as selective agents on a flower, relative to other pollinators. Pollinator importance has also been used to quantify the relative strength of the positive effects a pollinator can have on the plant partner (Thomson 2003, Reynolds and Fenster 2008).

I studied the interactions between a specialized pollinating seed predator, *Hadena ectypa* (Noctuidae), and its host plant, *Silene stellata* (Caryophyllaceae). *Silene stellata* occurs sporadically in understory light gaps and open meadows and is visited nocturnally by both this pollinating seed predator moth and other moth co-pollinators. Because the two types of pollinators are equally efficient at pollen deposition (Reynolds et al. 2012), I can use pollinator visitation rates as a proxy for pollinator importance when comparing *H. ectypa* and co-pollinators. However, *H. ectypa* also reduces host plant reproduction through flower and fruit predation resulting in a net negative outcome for *S. stellata* (Reynolds et al. 2012). When *S. stellata* is at low density or isolated, the net outcome of its interaction with *H. ectypa* is unknown but may differ from the outcome in large, continuous areas due to the potential benefit of the specificity of *H. ectypa* vs. generalist co-pollinators of *S. stellata*. I expect *H. ectypa* to provide the majority of pollination service (benefit) for isolated *S. stellata* plants because of their obligate dependence on *S. stellata* as a host plant for their young. With similar visitation between non-isolated and isolated plants, I expect predation rate (cost) also to be similar.

Specifically, I asked: Does the cost or benefit of pollination by specialized pollinating seed predators change when host plants are isolated? I quantified cost in terms of predation rates and benefit in terms of pollinator importance. To determine if pollinator importance of *H. ectypa* increased for isolated plants, I observed pollinator visitation and

quantified pollen deposition in small isolated and non-isolated patches of plants. Results from the first year of study led me to continue monitoring pollinator visitation to the non-isolated and isolated areas and to examine seed set, oviposition and predation level on individual plants in the field in areas with and without naturally occurring resident *S. stellata* plants (non-isolated and isolated areas) in the following year. To my knowledge, this is the first comprehensive examination of a pollinating seed predator-host plant interaction for plants in non-isolated and isolated settings.

Methods

Study Organisms—*Silene stellata* is a long-lived, iteroparous plant that occurs in open meadows or closed canopy woods across the Eastern half of the United States. Flowers are approximately 30 mm in diameter, protandrous and have fringed, white petals. Ovaries contain a mean of 25 ovules (Reynolds et al. 2009). Flowering frequently begins in early July at my study site and ceases in early September. *Silene stellata* is able to set seed autonomously (Reynolds et al. 2009) but exhibits the typical nocturnal moth pollination syndrome as anther dehiscence, stigma receptivity and scent production occur at dusk, and indeed, nocturnal moths are the primary pollinators (Reynolds et al. 2009). Of the moth pollinator community, *H. ectypa* is a specialized pollinator and seed predator of *S. stellata*. After nectaring, female *H. ectypa* may oviposit, most typically at the base of the ovary inside the large and persistent calyx. Eggs adhere to the ovary or inside calyx wall and can be counted reliably after fruit collection from the field. The larvae feed on flowers and fruits, each one consuming approximately 30–40 flowers or fruits before pupation in the lab (Reynolds et al. 2012). In almost all cases, the flower or fruit is

consumed completely with no ovules or seeds remaining. Moth co-pollinators in three families (Arctiidae, Noctuidae and Notodontidae) pollinate but do not oviposit on *S. stellata* flowers (Reynolds et al. 2009), and co-pollinators are as effective at pollen transfer as *H. ectypa* (Reynolds et al. 2012).

Study Sites—My study site is near Mountain Lake Biological Station (Giles County, Virginia, USA) and consists of a large, continuous population of *S. stellata* that naturally occurs in a meadow within a power line cut (~45 m wide) (37°20’53”N, 80°32’41”W, elevation ≈ 1,100–1,300 meters) where density is approximately 1.5 plants/m^2. This is the only dense population of this size within 10 km, dictating an experimental approach for comparing the outcome of the interaction with *H. ectypa* on non-isolated and isolated *S. stellata*. For this study, this meadow is the non-isolated area (Fig. 1). The isolated-woods areas occur in closed canopy woodland adjacent to both sides of the meadow, and I have not found *S. stellata* growing there, although the habitat is similar to wooded habitat nearby where *S. stellata* is established (but at lower densities than the meadow site). Finally, the isolated-meadow area is below a paved road (and its shoulders) that perpendicularly bisects the same power line cut but within a large area where *S. stellata* does not occur. The non-isolated meadow area and isolated-meadow area occur in the same meadow habitat and differ only in their location on the mountainside (nearly adjacent but separated by a road) and the presence of naturally occurring *S. stellata* plants; the surrounding plant community and other habitat aspects are the same in the two areas. The isolated-woods area was included to increase the generality of the study across habitat types and differs from the meadow areas in both the surrounding plant community

and light availability. The mean light level in the isolated-woods area was only 4 percent of the mean light level in the open meadow area (Isolated-woods: 132.6 μmol·s-1·m-2, meadow: 3516.9 μmol·s-1·m-2). All light levels were collected in the afternoon under partly cloudy conditions using the Pocket Light Meter, Version 6 (Nuwaste Studios) iPhone application and converted from lux. No other nocturnally flowering species occur at any of the three study areas within this site.

Experimental Design and Data Collected—I used potted plants to quantify the effect of isolation on pollinator visitation, pollen transfer, seed set, and *H. ectypa* oviposition and predation. Plant isolation was defined by a distance of 30 m from the nearest *S. stellata* plant because it is 10-fold greater than the mean interplant flight distances of moths visiting *S. stellata* (Reynolds et al. 2009). To generate the potted plants utilized here, *S. stellata* seeds were haphazardly collected from plants growing within the large, continuous population (the non-isolated area for this study) in 2006. After germination, plants were grown in the greenhouse until July 2008 and from September 2008 through July 2009 with ambient temperature and light and watered as needed (with four months outdoors each winter). Minimal maintenance of potted plants was required in both years. Plants were watered by hand if no rain had fallen in the previous five days or if the soil in the pot showed evidence of drying. Because all non-isolated and isolated study plants were potted in the same soil and water moisture levels were maintained at similar levels at the greenhouse and in the field, belowground resources should not differ among plants in the different areas. All potted plants in the three study areas were caged with 2-inch chicken wire for individual plants or netted exclosures for patches of plants to prevent

deer herbivory but allow natural pollination, fruit set, fruit maturation and predation by insects. I protected the plants against deer herbivory because of my specific interest in the effects of the pollinating seed predator on plant reproduction in the non-isolated and isolated areas. Within one week of experimental set up in 2009, significant disturbance by black bears displaced three individual potted plants within the isolated-meadow site transects. These three plants were replaced with fresh potted plants.

Because specialized *H. ectypa* pollinating seed predators and generalist co-pollinators are equally efficient at pollen transfer (Reynolds et al. 2012), I am able to quantify whether *H. ectypa* increases in pollinator importance relative to co-pollinators by documenting pollinator visitation by *H. ectypa* or moth co-pollinators in non-isolated and isolated patches of *S. stellata* plants. I observed patches of 5–12 potted plants on nights from 11–25 July 2008 (11 nights) and 13 July–4 August 2009 (19 nights). Occasionally weather (rain or cold temperature) precluded observations: it was unlikely that pollinators would visit on those nights (personal observation). Observations began after sunset (~8:45 pm E.S.T.), and the observation period each night was one hour and 45 minutes in 2008 and approximately one hour in 2009, corresponding to the period when most moth activity occurs (Reynolds et al. 2009). Pollinator type (*H. ectypa* or moth co-pollinator) was recorded for each moth pollinator visit. Moths do not discriminate between flowers of potted or naturally occurring plants (personal observation). Non-isolated and isolated patches were observed simultaneously. In 2008, infrared camcorders (Sony Digital Handycams, model TRV17) using the night vision setting supplemented personal observations in both the non-isolated and isolated patches and were fixed on groups of 3–10 flowers on three plants per patch each night. For the isolated patches in 2008,

observation nights alternated between the isolated-woods (N=6 nights) and isolated-meadow (N=5 nights). In 2009, for the last six observation nights, observations in the isolated areas alternated between the isolated-woods and isolated-meadow with three observations in each area, and camcorders were used in the non-isolated area for those six nights. In both years, total number of flowers in a patch was counted prior to the start of observations each night and ranged from 49–201 flowers per patch in 2008 and 7–50 flowers per patch in 2009. In 2009, there were fewer flowers per patch compared to 2008 because observations took place over a longer period of the season, until the last of the individual plants initiated flowering. Visitation rate was calculated from number of visits by a pollinator type divided by the number of flowers in the patch on an observation night divided by the length of the observation period. Before ANOVAs were performed, the visitation rate of each moth type in each patch on each night was square-root transformed. I analyzed the main effects of isolation and pollinator type and their interaction on the number of moths per flower per hour in each year.

To determine if the moths were equally effective in transferring pollen to isolated and non-isolated plants, I quantified stigmatic pollen loads in the non-isolated and isolated patches. Flowers were collected during the evening pollinator observation periods from the pollinator observation patches in 2008. After a single pollinator visit to a virgin female flower (first night as female), type of visitor (*H. ectypa* or co-pollinator) was recorded, the flower was placed in a glass tube, and samples were transported to the lab where the stigmas were removed then fixed and stained in glycerine jelly with fuchsin on a microscope slide (Beattie 1971). Stigmas from unvisited flowers (as observed during the observation period) were fixed and used as baseline controls. Stigmatic pollen loads

were counted using a compound microscope at 40x magnification. Before analysis, stigmatic pollen loads were $\log_{10}+1$ transformed. I performed ANOVA on the main effects of isolation and visitation and their interaction on $\log_{10}$ number of pollen grains per flower.

To further investigate outcomes in the interactions between *H. ectypa* and isolated *S. stellata*, individually placed potted plants were used to determine the effects of isolation on seed set per mature fruit, oviposition and predation by larvae in 2009. (In 2008, the focus on pollen deposition precluded the examination of these traits.) Eighteen individual potted plants were placed into each of the three areas along transects at 30 m intervals on 11–12 July 2009 (Appendix). In the non-isolated area, two parallel 270 m transects of 9 plants each were separated by 30 m, and these plants were an average of 1.1 m and 1.9 m from their nearest and second nearest naturally occurring neighbors. For the isolated-woods area, a 270 m transect was established on each side of the continuous population, 30 m into the woods and away from naturally occurring *S. stellata* plants in the non-isolated area. In the isolated-meadow three 180 m transects 30 m apart were established with six plants 30 m apart per transect. This arrangement of potted plants allowed me to maximize replication within the three study areas while maintaining the spatial separation among plants that was required to test my hypothesis. Number of flowers on the potted plants did not differ among the three areas.

On individual plants deployed in 2009, all flowers and fruits were collected ~21 days following flowering (3 August–9 September) when fruits were mature but prior to fruit dehiscence. Three plants in the isolated-woods area died prematurely, for a final sample size of 15 plants in that area, compared to 18 in the non-isolated and isolated-meadow

areas. Fruits were processed in the lab using a dissecting scope at 15x magnification. The following data were recorded for each plant: number of flowers that did not form a fruit, number of mature fruits, number of seeds found in mature fruits (without herbivory), number of egg cases on fruits or uneaten flowers and whether there was predation by *H. ectypa* larvae.

In order to calibrate the ability of moths to act as pollinators to isolated and non-isolated plants, I also quantified seed set from plants where no pollinator visitation was allowed to occur (pollinator exclusion plants). Twelve naturally occurring plants of similar size to my potted plants were caged with fine mesh screen and chicken wire to exclude pollinators prior to anthesis within the non-isolated field site and to prevent deer herbivory. Cages were removed from each plant after all fruits were collected. Light availability under cages in the meadow site was 72 percent lower than the level of light available for uncaged plants in the meadow (Caged: 966.5 μmol·s-1·m-2, Uncaged: 3516.9 μmol·s-1·m-2).

Seed set per mature fruit was calculated as the number of seeds per number of mature uneaten fruits for non-isolated, isolated-meadow, isolated-woods and pollinator exclusion plants. Nineteen isolated plants (isolated-meadow=6, isolated-woods=13) did not produce any mature, uneaten fruits and were therefore eliminated from the analyses. Seed set values were $\log_{10}$ transformed for ANOVA.

Oviposition and predation were calculated for non-isolated, isolated-meadow and isolated-woods plants. For each plant, oviposition was calculated as the number of eggs per number of fruits or uneaten flowers; I excluded the number of eaten flowers in this calculation because when flowers were eaten, the ovary was completely consumed

precluding egg counts. Due to extreme nonnormality of the residuals, oviposition rate was analyzed with a Kruskal-Wallis nonparametric test (Proc NPAR1WAY, SAS). Predation was calculated as the proportion of all flowers and fruits eaten per plant. Predation was square transformed for ANOVA.

All statistical analyses were performed in SAS 9.2. All ANOVA tests were performed using Proc GLM. When post-hoc means separation tests were required, Tukey's HSD was employed (unless otherwise noted). For each dataset, there was no statistical difference between the two isolated areas at $P<0.05$, thus data from isolated areas were combined into a general isolation category (Table 1).

Results

Pollinator Importance—*Visitation.* In 2008, there was a significant interaction between isolation (non-isolated vs. isolated) and pollinator type (*H. ectypa* vs. co-pollinator) ($F_{3,40}=6.96$, $P=0.0118$), with the highest visitation by *H. ectypa* to the non-isolated area, followed by *H. ectypa* visitation to isolated areas (Table 2). Co-pollinator visitation was significantly lower than *H. ectypa* visitation to both non-isolated and isolated patches. *Hadena ectypa* was, therefore, the most important pollinator in non-isolated and isolated areas.

In 2009 there was a significant difference in visitation rate (pollinator importance) of *H. ectypa* and co-pollinators ($F_{3,98}=0.31$, $P=0.0009$). *Hadena ectypa* visitation was higher than co-pollinator visitation over both non-isolated and isolated patches (Table 2). The main effect of isolation ($F_{3,98}=0.44$, $P=0.5071$) and the interaction between isolation and pollinator type ($F_{3,98}=0.31$, $P=0.5797$) were not significant.

Pollen deposition. Samples from a total of 102 flowers were collected (n=76 non-isolated, n=26 isolated). There was no effect of *H. ectypa* behavior (nectaring only vs. nectaring + ovipositing) on pollen deposition, and samples from these two types of visits were combined within non-isolated and isolated samples for further analysis ($F_{1,87}$=0.16, *P*=0.6876).

Only four samples from moth co-pollinator visits (all from *Autographa precationis*, Noctuidae) were collected due to low visitation rates. These samples were not included in the analyses: non-isolated: N=1, 23 pollen grains; isolated-meadow: N=3, mean=9.33 pollen grains per flower. These values fall within the range of stigmatic pollen loads resulting from *H. ectypa* visits (see below).

There was a significant interaction of visitation (visit by *H. ectypa* vs. no visit) and isolation (non-isolated vs. isolated) on stigmatic pollen loads ($F_{3,98}$=12.82, *P*=0.0005) (Fig. 2). Comparing flowers visited by *H. ectypa*, stigmatic pollen loads were three times higher in the non-isolated patch compared to the isolated patches. In the non-isolated patch, flowers visited by *H. ectypa* had higher stigmatic pollen loads than unvisited flowers. However, in the isolated patches, stigmatic pollen loads were not different between flowers visited by *H. ectypa* and unvisited flowers. As expected, stigmatic pollen loads on unvisited flowers were low and did not differ between non-isolated and isolated patches. Main effects were not significant (isolation: $F_{1,98}$=0.66, *P*=0.4171; visitation: $F_{1,98}$=1.04, *P*=0.3100).

Seed set per mature fruit. Seed set per mature fruit was significantly greater for the non-isolated plants compared to the isolated plants and pollinator exclusion plants (Table 3, $F_{2,42}$=5.59, *P*=0.0070).

Oviposition. There was no effect of isolation on egg deposition for non-isolated versus isolated plants (Table 3, $\chi^2_{1,47}$=1.9984, *P*=0.1575).

Predation. Predation of flowers and fruits per plant was significantly greater for plants in the isolated areas compared to the non-isolated area (Table 3, $F_{1,49}$=4.11, *P*=0.048).

Discussion

In this examination of the interactions between a pollinating seed predator and isolated host plants, I found decreased benefit and increased cost of the specialized pollinator seed predator for its host plant, *S. stellata*, independent of the isolated plants' habitat. Because of its significantly higher visitation rates and equivalent pollinator effectiveness compared to moth co-pollinators (Reynolds et al. 2012), *H. ectypa* was the most important pollinator in both non-isolated and isolated patches. However, *H. ectypa* had lower pollinator effectiveness in isolation compared to the non-isolated area, and so its relative importance decreased in isolation. The lower stigmatic pollen loads resulted in lower seed set for plants in isolation. Furthermore, despite similar oviposition rates between the two areas, predation was greater for plants in isolation.

Seed set of isolated and pollinator exclusion plants was similar, a pattern consistent with the lower stigmatic pollen loads experienced by isolated plants compared to non-isolated plants. Stigmatic pollen load differences were likely driven by lower pollen availability in isolated patches (Knight et al. 2005). For isolated single or small groups of plants, scant pollen transfer from nearby continuous natural population could result in pollen limitation. Although pollen dispersal distances of tens of meters have been

reported for *Silene latifolia* (as *Silene alba*) (McCauley 1997, Richards et al. 1999), nocturnal moth pollinators of *S. stellata* moved fluorescent dye (as a pollen analog) only 2.2 m on average (Reynolds et al. 2009). A fluorescent dye study of *S. latifolia* (as *S. alba*) also revealed short average pollinator flight distances of 1.2 m for moths (Young 2002). It is therefore not surprising that *H. ectypa* moths are inefficient pollinators of *S. stellata* when initially traveling 30 m from a dense patch of plants to a small, isolated patch. It is also possible that *H. ectypa* adults flew to isolated areas and repeatedly visited plants in isolation, quickly depleting the limited supply of pollen in isolated patches, rather than returning to the non-isolated areas where pollen availability was much greater due to the surrounding naturally occurring plants (Schulke and Waser 2001).

Similar stigmatic pollen loads on unvisited and visited flowers in the isolated patches indicates that pollinator-mediated pollen deposition made only small (if any) contributions to stigmatic pollen loads in isolated patches. Although I do not have experimental confirmation of pollen limitation of seed set, it is likely that seed set differences between non-isolated and isolated patches are attributable to lower pollen deposition into and within the isolated areas, rather than resource limitation, because fruit initiation was consistent throughout the entire flowering season which is contrary to the expectations of resource limitation (Knight et al. 2005). Additionally, the seed set data come from individually deployed plants that likely would suffer even lower levels of pollinator visitation and stigmatic pollen loads compared to the small patches of plants I used for pollinator observation and pollen deposition sample collection. Although pollen deposition data were collected in year 1 and seed set data were collected in year 2, pollinator efficiency does not change from year to year when there is similar availability

of pollen donors and similar visitation rates (Reynolds et al. 2009).

The ultimate cost of pollination by pollinating seed predators is loss of seeds through flower and fruit predation (plus loss of male reproductive function through predation of male phase flowers). The cost of predation may be mediated through oviposition patterns because egg laying initially determines the number of larvae potentially present on a plant. In my experiment, oviposition was similar between non-isolated and isolated areas (corresponding to the similar visitation rates of *H. ectypa* to non-isolated and isolated areas in 2009, Reynolds et al. 2012), which is in contrast to results of Elzinga et al. (2005), who found higher oviposition in isolated populations. They give two reasons for the pattern that might be relevant to my results: 1) in low density patches the decision to leave a host plant could be altered and 2) the acceptance of lower quality flowers could increase. Their small, isolated experimental plots, however, contained 16 plants compared to the single plants used here, and their patches were set apart at 100 m versus 30 m from the dense population in this design. The closer proximity of my isolated plants in relation to non-isolated plants means that *H. ectypa* moths could visit isolated patches with less energy required but then more easily return to the closer higher density area after ovipositing. Because I used potted plants of similar phenology and number of flowers, it is unlikely moths detected any differences in flower quality between my non-isolated and isolated potted plants.

Unlike oviposition, mean plant predation was significantly higher in isolated areas: 66% of total flowers in isolation vs. 54% for non-isolated plants (untransformed means). Furthermore, the maximum level of predation was higher in isolated areas: no plant in the non-isolated area had 100% predation compared to seven out of 33 isolated plants. Only

two of 18 non-isolated but 20 of 33 isolated plants suffered a greater than 80 percent predation rate. There are several possible mechanisms for the negative relationship between plant density and predation.

When *S. stellata* plants have fewer than 30 flowers or fruits, larvae will be food limited (Reynolds et al. 2012, under lab conditions). Furthermore, larvae prefer younger flowers before the fruits become hardened (Castillo et al., In preparation, under lab conditions). Larvae on plants in the non-isolated area would be able to move to nearby naturally occurring plants with available flowers and fruits that might be of higher quality (younger, softer) whereas larvae on the isolated plants had no plants within 30 m to move to additional resources. While 30 m seems possible for adult moths to fly, 30 m is a long distance for larvae to travel (Hagstrum and Subramanyam 2010). Consequently, larvae on isolated plants may have consumed less favorable fruits because no other suitable plant material was available nearby (Kunin 1997).

Another possible explanation for lower seed predation in the non-isolated area is potentially higher natural enemy abundance in the established or high density area such that isolated areas are enemy-free spaces (Jefferies and Lawton 1984). Although I have casually observed parasitoids emerge from *H. ectypa* larvae in the lab, I was not able to collect data on parasitism rates of *H. ectypa*. However, other studies of *Silene* pollinating seed predators shed light on possible parasitism rates. Parasitism can be high (nearly 50 percent and involving 13 parasitoid species) with decreased parasitoid presence in smaller patches and lower parasitism rates of *Hadena* in smaller, more isolated populations (Elzinga et al. 2003, Elzinga et al. 2005, Elzinga et al. 2007a). Parasitoids can have profound impacts on larval consumption: *Hadena* larvae consumed 50% less

plant material when parasitized, even though they were parasitized by a koinobiont parasitoid that allows for continued larval feeding (Elzinga et al. 2003).

Contrary to my hypothesis, isolated plants did not benefit from increased pollination by specialized pollinating seed predators. Although pollinator importance of *H. ectypa* was higher than co-pollinators in both non-isolated and isolated patches, stigmatic pollen loads after a visit by *H. ectypa* were lower for isolated plants thereby resulting in decreased benefit of *H. ectypa* for plants in non-isolated vs. isolated patches. Consequently, plants in isolated areas produced no more seeds per mature fruit than my pollinator exclusion plants. Without pollinating seed predators, isolated plants may have the chance to produce a low number of seeds from an occasional visit by a non-specialist co-pollinator or within-flower self pollination (like my caged pollinator exclusion plants). Self pollination, however, may result in inbreeding depression and have important consequence on seed number and quality (Dudash 1990, 1991).

Reduced reproductive output in isolated areas indicates that isolated *S. stellata* plants may experience a component Allee effect for seed set. There are two possible causes of this component Allee effect for *S. stellata*: reduced pollination and greater seed predation. Jules and Rathcke (1999) also found a similar Allee effect due to multiple causes, but evidence for multiple causes of component Allee effects is uncommon (Berec et al. 2007, Kramer et al. 2009).

A component Allee effect through reduced seed set in isolation may not be immediately noticeable for long-lived herbaceous plants (such as *S. stellata*) that may be less dependent on annual seed production for population persistence. For plants that can reproduce both sexually and asexually, however, it is the sexually produced offspring that

are important in population establishment (Ceplitis 2001), and seed predation has greater impacts for plants on the edge of an expanding population or cut off from the main population through habitat fragmentation compared to plants within established populations (Harper 1977). In this pollinating seed predator system, however, overcoming an Allee effect due to reduced pollination and greater seed predation is complicated because the pollinators that are most frequent and could alleviate pollen deficits through increased visitation are not effective pollinators for isolated plants, and their young are the seed predators. If this component Allee effect translates into a patch-level effect on fitness, then a demographic Allee effect may be observed (Stephens et al. 1999), thereby demonstrating the consequence of isolation for population expansion and persistence and indicating the importance of considering species interactions in conservation of populations of endangered and threatened species.

Differences in the ecological setting of an interaction can result in variation in the outcomes of interaction (Bronstein 1994). For example, over broad temporal and spatial scales interaction outcomes may vary (Billick and Tonkel 2003). Others have shown effects of herbivores on host plant demography over large-scale environmental gradients (Louda 1982, Rand 2002, Miller et al. 2009) and between population differences in outcomes of interactions resulting in a geographic mosaic of coevolution (Thompson and Cunningham 2002, Thompson and Hernandez 2006, Thompson et. al 2010, Reynolds et al. 2012). However, my study focuses on interactions at a local geographic scale to show that varying ecological context even within a population can affect the outcomes of interspecific interactions at a relatively small scale (10s of meters). For pollinating seed predator interactions, no other study has fully demonstrated this phenomenon by

examining pollinator frequency and efficiency and seed set per mature fruit along with oviposition and predation for non-isolated and isolated plants.

Tables

Table 1. ANOVA results (F values, degrees of freedom and P values) comparing data from isolated-meadow and isolated-woods areas for pollinator visitation (2008 and 2009), stigmatic pollen loads (2008), seed set per mature fruit (2009), oviposition (2009) and predation (2009). Visitation rate was calculated from number of visits by a pollinator type divided by the number of flowers in the patch on an observation night divided by the length of the observation period. Oviposition was calculated as the number of eggs per number of fruits or uneaten flowers, and predation was calculated as the proportion of all flowers and fruits eaten per plant.

Pollinator Visitation	F	DF	*P*
2008	1.28	20	0.2719
2009	3.04	62	0.0864
Stigmatic Pollen Load	0.02	24	0.8797
Seed Set	1.75	14	0.2067
Oviposition	1.59	29	0.2169
Predation	0.02	31	0.8921

Table 2. Untransformed means and 1 standard error (S.E.) for visitation rate of *H. ectypa* and co-pollinators in 2008 and 2009 in non-isolated and isolated patches. Visitation rate was calculated from number of visits by a pollinator type divided by the number of flowers in the patch on an observation night divided by the length of the observation period. Because habitat type had no effect on visitation to isolated patches, I pooled the isolated patches and present one mean. Different letters in the right side column represent significant differences at $P < 0.05$ within each year.

2008	Visitation rate	S.E.	
Non-isolated - *H. ectypa*	0.243	0.028	a
Non-isolated - co-pollinators	0.015	0.004	c
Isolated - *H. ectypa*	0.135	0.027	b
Isolated - co-pollinators	0.019	0.005	c
2009	Visitation rate	S.E.	
Non-isolated - *H. ectypa*	0.246	0.072	x
Non-isolated - co-pollinators	0.061	0.020	y
Isolated - *H. ectypa*	0.291	0.059	x
Isolated - co-pollinators	0.057	0.015	y

Table 3. Untransformed means and 1 standard error (S.E.) for plant reproductive measures taken on individually potted plants in 2009. Oviposition was calculated as the number of eggs per number of fruits or uneaten flowers, and predation was calculated as the proportion of all flowers and fruits eaten per plant. Because habitat type had no effect on seed set, oviposition and predation for isolated plants, means are pooled across the two habitat types for isolated plants. Different letters in the right side column represent significant differences at $P < 0.05$ for each dataset.

Seeds per fruit	Mean	S.E.	
Non-isolated	14.497	2.222	a
Isolated	7.040	1.875	b
Pollinator exclusion	6.537	1.488	b
Oviposition	Mean	S.E.	
Non-isolated	0.152	0.040	x
Isolated	0.132	0.039	x
Predation	Mean	S.E.	
Non-isolated	0.544	0.054	g
Isolated	0.661	0.055	h

Figures

Figure 1. Schematic diagram of the *Silene stellata* field site. Closed circles represent the general placement of individual potted plants used for fruit set, seed set, egg laying and predation data collection in 2009. Open circles represent the general location of sets of potted plants placed in the field for pollinator observations in 2008 and 2009 and flower collection for pollen deposition data in 2008. Gray-filled areas on the sides of the diagram represent wooded area, while the center area is all open meadow created by a power line cut. The stippled area at the top of the diagram represents the area with a continuous population of naturally-occurring plants.

Figure 2. Stigmatic pollen loads from flowers collected from individual potted plants in the non-isolated and isolated patches in 2008. Because habitat type had no effect on stigmatic pollen loads of isolated plants, means are pooled across the two habitats. The means and standard errors presented are untransformed, however, statistical analyses were performed on $\log_{10}$ transformed data. Error bars represent ± one standard error. Different letters above the bars represent significant differences of transformed means.

Figure 1.

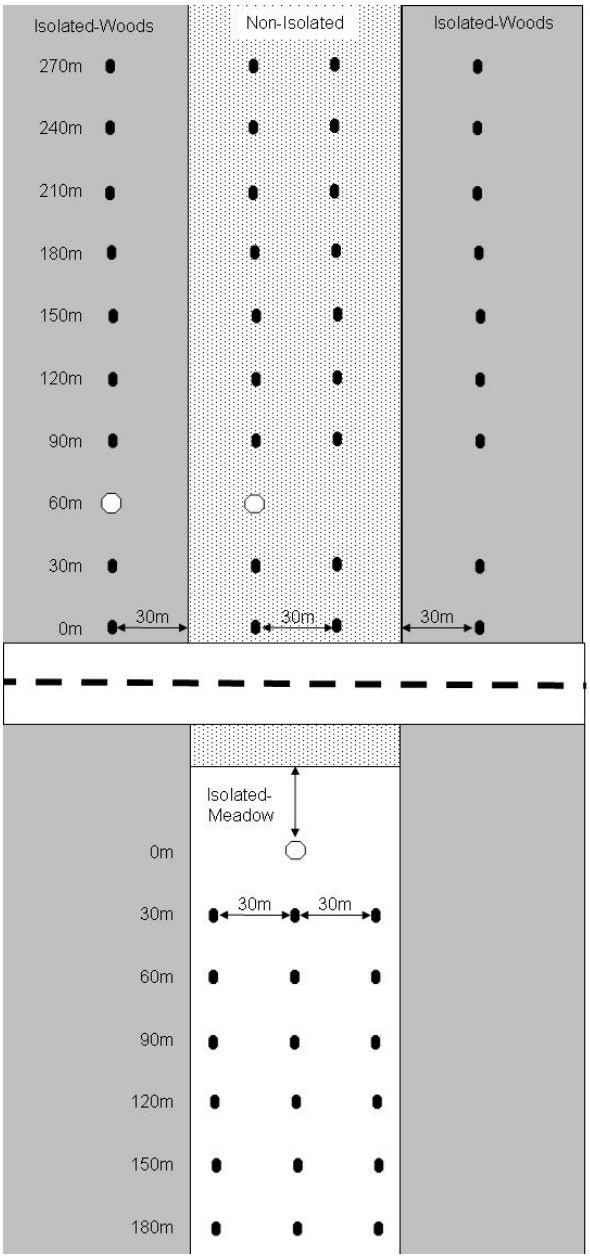
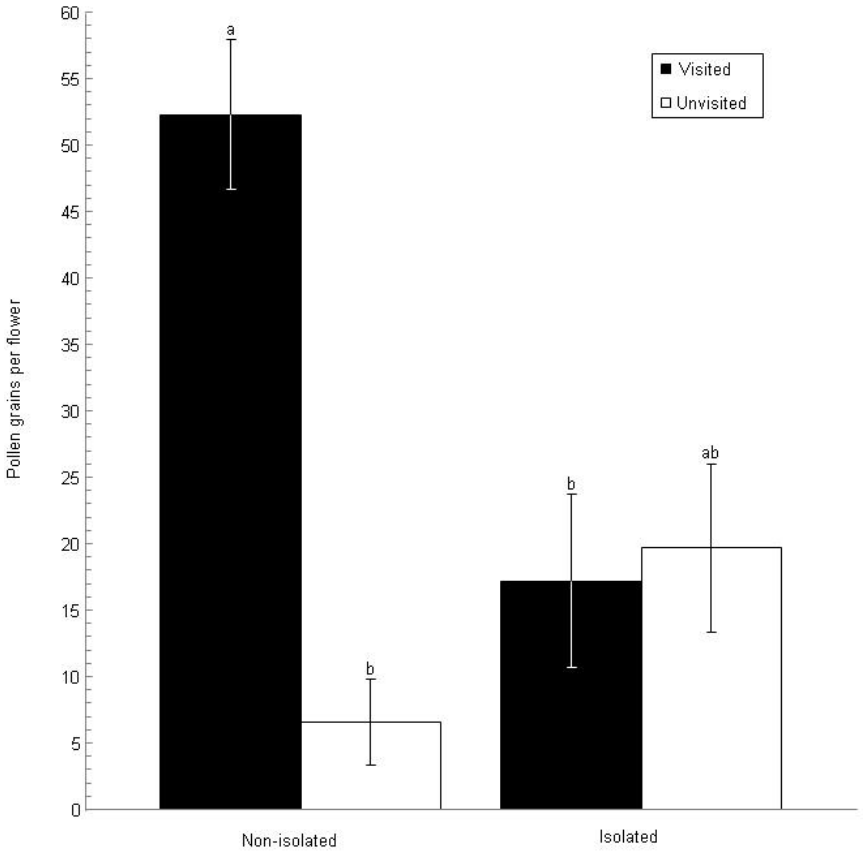

Isolated-Woods
Non-Isolated
Isolated-Woods
270m
240m
210m
180m
150m
120m
90m
60m
30m
0m
30m
30m
30m
Isolated-
Meadow
0m
30m
30m
30m
60m
90m
120m
150m
180m

Figure 2.


Visited
Unvisited
Pollen grains per flower
60
55
50
45
40
35
30
25
20
15
10
5
0
a
b
b
ab
Non-isolated
Isolated

Chapter 3

Host plant synchrony with its pollinating seed predator contributes to the interaction outcome between *Silene stellata* (Caryophyllaceae) and *Hadena ectypa* (Noctuidae)

Abstract

Phenology plays an important role for plant-insect interactions, and to understand the combined effect of flowering and pollinator or florivore phenology on species interaction outcomes, synchrony between flowering and pollinator or florivore activity within and across seasons are needed. To my knowledge, this study on the facultative interaction between *Silene stellata* and *Hadena ectypa* is the first to quantify a measure of synchrony between host plants and pollinating seed predators. I observed moth pollinator visitation, determined stigmatic pollen loads, followed the timing of flowering and *H. ectypa* oviposition and larval density, then determined initiated fruit set and flower and fruit predation rates on focal study plants across two flowering seasons. I calculated synchrony between focal plant flowering and pollinating seed predator oviposition. Pollinator visitation, stigmatic pollen loads and larval density per plant varied significantly within at least one season, however, none of the variables were different between years. Oviposition decreased across the season in both years and was significantly higher in 2009. Flowering density within the population was significantly greater in 2008 and increased across 2008, while it decreased across 2009. Initiated fruit set was similar

between years, and predation was higher in 2009. The magnitude of synchrony between *S. stellata* flowering and *H. ectypa* oviposition was significantly higher in 2009 than in 2008. Synchrony did not affect initiated fruit set (an indication of pollination) in either year. However, synchrony had a significant effect on predation in both years but in opposite directions: plants with highest synchrony levels in 2008 had the highest levels of predation, whereas in 2009 plants in highest synchrony with oviposition experienced the lowest predation. Interannual variation in the magnitude of synchrony, as well as, potential climatic differences may explain the contrasting relationship between synchrony and predation between years. This study identifies a specific scenario (high levels of synchrony) in which facultative pollinating seed predators may have a mutualistic outcome with their specialized host plant.

Introduction

Phenology plays an important role in the outcome of plant-insect interactions (Rathcke and Lacey 1985, Crawley 1997, Kearns et al. 1998). Because host plants and insects may use different cues to determine phenological timing (van Asch and Visser 2007) or may respond differently to climate changes (Parmesan and Yohe 2003), a mismatch in flowering and insect activity may occur and result in either positive or negative effects on host plant fitness depending on whether the interaction is primarily mutualistic or antagonistic (Harrington et al. 1999, Hegland 2009, Forrest and Miller-Rushing 2010). For instance, phenological mismatches with pollinators could cause reduced host plant reproduction (Hegland et al. 2009), whereas mismatches with herbivores may result in higher fitness for host plants (Collin and Shykoff 2010). Therefore, the degree of

synchrony between flowering and pollinator and florivore activity within a season should be calculated to understand the combined effects of phenology of flowering and pollinator or florivore activity on interaction outcomes (Russell and Louda 2004).

The potential impact of phenological matches or mismatches between host plants and pollinators and insect herbivores has been the subject of multiple recent reviews (Russell and Louda 2004, Elzinga et al. 2007b, Miller-Rushing et al. 2010), however, few studies actually quantify plant-insect synchrony (Russell and Louda 2004 and references within, Russell and Louda 2005, van Asch and Visser 2007). When it is calculated, synchrony usually is determined at the population level, which, if data are collected over many years and populations, may provide an explanation for spatiotemporal variation in species interactions (e.g., Louda et al. 2004, Singer and Parmesan 2010). However, quantifying among year variation in synchrony is not sufficient for studying the outcomes of interactions within a season (Hegland et al. 2009). Monitoring the temporal distribution of individual phenology (rather than population-level monitoring using metrics such as date of first flower or first arrival) facilitates the construction of individually-based synchrony measures that allow for a better understanding of host plant vulnerability to phenological matches or mismatches with pollinators or antagonists within a season (Miller-Rushing et al. 2010). In particular, calculating synchrony at the individual level allows translation of the consequences of variation in synchrony into reproductive differences and potentially provides a mechanistic understanding of fitness differences among individuals within a population.

In pollinating seed predator mutualisms (a.k.a. nursery pollination), pollinating seed predator adults first pollinate flowers, and then females lay eggs on the flowers (Wiebes

1979, Dufay and Anstett 2003, Kephart et al. 2006). After hatching, larvae feed on flowers, fruits and/or seeds. Synchronization between partners may favor the persistence of these mutualistic interactions (Addicott et al. 1990, Bronstein 1992). However, poor matching in mutualisms has been documented (Ollerton and Lack 1992, Herrera 1998), and recent theoretical research suggests that high levels of synchrony between plants and pollinating seed predators should not be expected because of conflicting selection on phenology via the mutualistic and antagonistic effects of pollinating seed predators on host plant reproduction (Brody 1997, Law et al. 2001). Indeed, Elzinga et al. (2007) found that the effects of pollinators and antagonists on selection for flowering phenology often are in opposition.

For highly obligate relationships (e.g., fig-fig wasp, yucca-yucca moth), host plant and insect phenology have likely coevolved (Law et al. 2001), and therefore may not be ideal study systems to examine the role of synchrony in determining interaction outcomes. Whereas for facultative pollinating seed predator interactions (e.g., *Silene-Hadena*), coevolution of tightly matched host plant and pollinating seed predator phenology may not be as likely due to positive interactions between host plants and co-pollinators that do not use the flowers and fruits as larval food resources (Kephart et al. 2006). In both types of pollinating seed predator systems, plant flowering phenology must match pollinating seed predator adult activity in order to receive their pollination services (Bronstein 1992), but it is more difficult to determine whether plants within a population that are synchronous or asynchronous with their pollinating seed predators will suffer the highest levels of seed predation because of the temporal separation between pollination and seed predation.

The role of *Silene* (and allied) spp. flowering phenology on outcomes of pollinating seed predator interactions with *Hadena* spp. has been documented. Pollination may be similar for plants flowering across the season (Pettersson 1991) with predation rates higher for earlier plants (Pettersson 1991, Biere and Honders 1996), or fruit set may decrease while predation increases across the season (Biere and Honders 1996, Collin and Shykoff 2010). Between or within year variation in levels of predation may be the result of higher density (and therefore, visitation rates) of *Hadena* in different years or at different times of the season (Pettersson 1991, Biere and Honders 1996), but these potential effects of pollinating seed predator phenology have not been quantified. Determining not only the phenology of *Silene* flowering and *Hadena* activity, but also, their synchrony may shed light on whether this interaction is mutualistic or parasitic for the host plant (Bopp and Gottsberger 2004).

I quantified the phenology of *Silene stellata* flowering and its pollinating seed predator, *Hadena ectypa*, then calculated synchrony between individual plant flowering and oviposition to determine the effect of synchrony on host plant reproductive success. My goal was to determine whether host plant and pollinating seed predator synchrony across the season altered the interaction outcome within one population for two years. This study goes beyond previous work on pollinating seed predator mutualisms by collecting detailed data on both individual host plant flowering and also pollinating seed predator activity and then calculating a measure of synchrony between the two. First I documented the within season phenology of pollinating seed predator activity and host plant flowering by measuring moth visitation, stigmatic pollen loads, flowering density, and *H. ectypa* oviposition and larval density across two flowering seasons, and

additionally, I examined the interannual differences of these variables. Then I calculated host plant flowering-pollinating seed predator synchrony. I focused on within-season individual plant (rather than population-level) synchrony with pollinating seed predators and predicted that individual plants would experience different interaction outcomes depending on their synchrony with *H. ectypa.* I hypothesize that 1) plants that flower synchronously with pollinating seed predator adults may experience higher female reproductive output because of their overlap with the pollination activity portion of the interaction and limited overlap with larval predation, and 2) plants asynchronous with pollinating seed predator adults may be more negatively affected because they have overlap with larval activity and could be susceptible to flower and fruit predation without benefiting from pollination services.

Methods

Study organisms—*Silene stellata* is a native, long-lived, iteroparous perennial plant that occurs in the eastern part of the United States in forest or meadow habitats. The flowers are hermaphroditic and protandrous with white, fringed petals and exhibit a nocturnal pollination syndrome (Reynolds et al. 2009). A frequent pollinator is *Hadena ectypa* (Noctuidae). Adult *H. ectypa* nectar at flowers, then, on approximately 40% of all visits (Kula et al., Unpublished data), or nearly all visits by females, *H. ectypa* females oviposit into the corolla tube and lay an egg at the base of the ovary or on the inside of the calyx. Oviposition by *H. ectypa* is a good measure of pollinating seed predator adult activity because it is indicative of both pollination of *S. stellata* and the initial step toward seed predation (Reynolds et al. 2012, Chapter 1). Pollination during oviposition visits is

equivalent to visits in which *H. ectypa* nectar only (Chapters 1 and 2). Larvae feed on up to 40 flowers and fruits under laboratory conditions (Reynolds et al. 2012). Other nocturnal moth co-pollinators visit flowers to feed on nectar (Reynolds et al. 2012). *Hadena ectypa* and moth co-pollinators are equally effective at pollen transfer, thus their pollinator importance (= frequency x effectiveness, Reynolds and Fenster 2008) is dependent on their relative visitation rates (Reynolds et al. 2012). Aside from deer browsing, all flower and fruit consumption results from *H. ectypa* feeding.

Study site—I studied the interactions between *S. stellata* and *H. ectypa* in a clear cut meadow produced by a power line cut in the Allegheny Mountains of southwest Virginia near the University of Virginia's Mountain Lake Biological Station in Giles County, Virginia, U.S.A. (37°20'53"N, 80°32'41"W, elevation ≈ 1,100–1,300 meters). The flowering season at this site starts in early July and continues through early September.

Pollinators and pollination—*Pollinator observations*. To quantify pollinator abundance and community composition, I conducted pollinator observations from mid-July through mid-August when flower density is highest (Reynolds et al. 2012). On each sampling night, observations of the natural population began within one hour after sunset when moth pollinator activity is highest and continued through the highest activity period and therefore when most pollination occurs (Reynolds et al. 2009). During observations, I wore a headlamp with a red light to facilitate observations without disturbing moth pollinators. The starting point at the base of the mountain alternated each evening, and no two patches were observed on consecutive evenings. Censuses commenced within the

season when flowering density was high enough to accommodate observations on at least 20 patches of plants.

I observed moth pollinators in haphazardly selected patches of plants, and by moving 10–15 m from patch to patch, I avoided re-counting moths that might fly between plants during observations and captured an estimate of the moth community for each night within the entire field site. I examined all flowers within the patch for nocturnal moth pollinators contacting floral reproductive parts of *S. stellata*. Total number of flowers per patch was estimated from number of plants within a patch x number of flowers on a haphazardly chosen representative plant within the patch. The total number of *H. ectypa*, nocturnal moth co-pollinators and total nocturnal moth pollinators (=*H. ectypa* and co-moth pollinators combined) for each night was divided by the total number of flowers observed on that night (summed across patches) to obtain the number of total nocturnal moth pollinators per flower for the population on each night.

Observation periods lasted approximately 35–60 min. (mean=45 min. in 2008, 50 min. in 2009). Approximately 90 s was spent in each patch resulting in observation of each flower for just an instant (~2 s in 2008 and ~3 s in 2009), however, I was interested in quantifying the number of pollinator visits a flower experiences over the entire active period for each night. Therefore, number of moths per flower was scaled up to a total nighttime visitation rate by multiplying moths per flower by the number of 2 s (2008) or 3 s (2009) observations each flower would receive during the 45- and 50-minute observation periods in 2008 and 2009 (1500 in 2008 and 1000 in 2009). This product provides an estimate of the number of total nocturnal moth visits to a flower during the period of highest pollinator activity.

Stigmatic pollen loads. To determine if pollinator effectiveness varied throughout the flowering season, one first day female flower was collected from up to 15 haphazardly chosen plants from across the site each morning after the nighttime pollinator observations, and collections were made prior to high diurnal pollinator activity (before 7:30 am). Flowers were transported to the laboratory where stigmas from collected flowers were permanently fixed on microscope slides with fuchsin-stained glycerin jelly (Beattie 1971), allowing later quantification of stigmatic pollen loads using a compound microscope with 40x magnification. Additionally, five flowers were collected in the evening prior to the start of pollinator activity as unvisited controls. The mean stigmatic pollen load from each set of control samples was subtracted from the next morning's flowers stigmatic pollen load, and these adjusted stigmatic pollen loads were square-root transformed for analysis.

Focal plant observations—To determine flowering phenology, oviposition rates and larval density on dates across the flowering season, as well as, the outcome of the interaction between *S. stellata* and *H. ectypa*, I monitored 118 and 96 plants in 2008 and 2009, respectively, as focal study plants. The plants were originally flagged within the study site between 2003 and 2005. In 2008 and 2009, 34 and 43 of the study plants, respectively, were naturally occurring, and 80 and 54 plants, respectively, were from a set of plants grown from seeds collected at the field site and transplanted to the field site in 2004 and 2005. I selected plants that represented the natural flowering phenology within the population. Plants were protected from deer browsing with caging material that allowed free movement of pollinators and insect herbivores. I chose to exclude deer

browsing because of my explicit interest in *H. ectypa* and its effects on *S. stellata*.

After flowering was initiated within the population, each focal plant was visited every 2–4 days, and at each visit all new and previously unmarked flowers were marked with uniquely colored labeling tape wrapped around the stem just below the flower. The number of new open flowers was counted by floral stage (male, first day with stigmas protruding = day 1 female, second day with stigmas protruding = day 2 female, older female). The total number of eggs in the set of newly marked flowers on each plant was counted using a hand lens to examine the ovary and inside calyx surface where eggs are laid. The number of larvae observed on each plant was recorded. Larvae were typically first observed after they had initiated movement between flowers, which is after the third instar for *H. bicruruis* on *S. latifolia* (Bopp and Gottsberger 2004), and when they were approximately 10–15 mm in length. At the end of the season, all flowers and fruits were collected using a standard set of collection criteria (e.g., color and hardness of fruit wall) and grouped by marking period for each plant. Plants were visited until all fruits were collected. Flowers and fruits were assessed within the laboratory for fruit initiation and predation by *H. ectypa* larvae using a dissecting scope with 15x magnification.

I calculated two metrics to quantify the consequences of interactions with *H. ectypa* for *S. stellata* focal plants for dates across the season for two years and differences between the two years. The proportion of initiated fruit (also referred to as initiated fruit set) was the number of flowers to set a fruit (both fruits with seeds and those eaten by *H. ectypa* larvae) divided by total flower production on an individual focal plant or for a marking date within the observation period. Predation was the number of flowers and fruits that were eaten divided by total flower production for an individual focal plant or

for a marking date within the observation period. Initiated fruit set, therefore, accounts for pollination success or total pollinator effect on potential reproductive success for an individual focal plant or marking date (unpollinated flowers and eaten flowers were excluded). Predation accounts for loss of potential reproductive success through flower and fruit consumption (unpollinated flowers and successful fruits were excluded) for an individual focal plant or marking date. Because these metrics were calculated as proportions, they were arcsine square-root transformed prior to analysis.

Synchrony—By closely monitoring the timing of flowering and moth abundance on the focal study plants within my study population throughout the 2008 and 2009 flowering seasons, I was able to calculate a measure of synchrony between flowering and moth oviposition activity for each focal plant. For an individual focal plant, I multiplied the proportion of flowers open on a marking date times the number of eggs per flower for that marking date. I then summed the products for each plant to obtain a plant-level synchrony measure that accounts for the degree of overlap with oviposition across the plant's entire flowering duration. Plants with synchrony=0 have no overlap with oviposition activity of *H. ectypa*. Plants with the highest levels of synchrony opened a high proportion of their flowers during the highest *H. ectypa* oviposition period (see Appendix A for example calculations).

Because I was explicitly interested in the role of synchrony between host plants and pollinating seed predators in their interaction outcomes, my measure of synchrony does not take into account the abundance of co-pollinators. Co-pollinators are similarly efficient at pollination compared to *H. ectypa* (Reynolds et al. 2012), however I found

that co-pollinators did not become a prominent component of the pollinator community until after 5 August 2008 at approximately six moths per flower and 1 August 2009 at approximately four moths per flower. By that time, larvae were active with approximately (or just under) one larva per plant and therefore likely consuming flowers that would be visited by co-pollinators.

Statistics—To examine relationships between day of the year (January 1=Day 1) and 1) pollinator visitation by *H. ectypa*, co-pollinators and total nocturnal moth pollinators; 2) flowers marked per day; and 3) oviposition and larvae per plant, I used nonparametric regression utilizing thin plate splines on the mean values for each date (Proc TSPLINE, SAS 9.2). This method has been commonly used in studies of climate and morphology (e.g., Hutchinson 1995, Price et al. 2000, Nattero et al. 2010) but has also been invoked in recent ecological studies where relationships are clearly complex (e.g., Reynolds et al. 2012).

I also used generalized linear models to test for relationships between day of the year and 1) pollinator visitation by *H. ectypa*, co-pollinators and total nocturnal moth pollinators; 2) flowers marked per day; and 3) oviposition and larvae per plant, in addition to, initiated fruit set and predation (Proc GENMOD, SAS 9.2). Stigmatic pollen loads were analyzed using a generalized linear mixed model with day as a random factor (Proc GLIMMIX, SAS 9.2). For both types of procedures, I specified a Poisson distribution with a logarithmic link function, except for initiated fruit set and predation for which I used a binomial distribution and a logistic link function.

I examined the relationships between plant synchrony and initiated fruit set and

flower and fruit predation by *H. ectypa* larvae using generalized linear models (Proc Genmod, SAS) with a binomial distribution and logistic link function.

Year to year differences in daily (or nightly) mean values of 1) visitation by *H. ectypa*, moth co-pollinators and total nocturnal moth pollinators; 2) flowers marked per day; and 3) oviposition and larvae per plant were analyzed using ANOVA (Proc GLM, SAS 9.2), and stigmatic pollen loads were analyzed using a mixed model ANOVA with day as a random factor (Proc MIXED, SAS 9.2). I used mixed model ANOVA with plant identification as a random factor to compare individual plant initiated fruit set, predation and level of synchrony between years (Proc MIXED, SAS 9.2).

Results

Pollinators and pollination—*Pollinator observations*. Observations took place on 22 nights in 2008 and 21 nights in 2009, and the mean number of patches observed each night was 30 in 2008 and 28 in 2009 with a mean of 8 plants per patch in both years.

The number of *H. ectypa* visits per flower decreased significantly over the season in both years (Table 1) (Figure 1). Peak visitation rate was 29 moths per flower in 2008 (21 July). Peak visitation was 24 moths per flower in 2009 (23 July). Moth co-pollinator visitation increased across the season in both years (Table 1) (Figure 1). Moth co-pollinator peak visitation rate was 11 moths per flower in both years, and the peak visitation occurred on 27 July 2008 and 16 August 2009. Similarly to *H. ectypa* (and because *H. ectypa* was the most frequent pollinator), total nocturnal moth visits decreased over the season (Table 1) (Figure 1), and dates of peak visitation rate were the same as for *H. ectypa* visitation (21 July 2008 and 23 July 2009). Nightly visits per flower by *H.*

ectypa, co-pollinators and total nocturnal moth pollinators did not differ between years at $P<0.05$ (Table 2).

Stigmatic pollen loads. In 2008, a total of 291 flowers were collected on 20 days from 24 July–22 August, and in 2009, 282 flowers were collected on 19 days from 23 July–19 August. Daily mean morning stigmatic pollen loads ranged from 8–308 in 2008 and 50–370 pollen grains in 2009. In 2008, stigmatic pollen load increased significantly throughout the season ($X^2_{1,18}=11.13$, $P=0.0037$). However stigmatic pollen loads did not vary across the 2009 flowering season ($X^2_{1,17}=1.07$, $P=0.3161$). Daily mean pollen load across the season did not differ between 2008 and 2009 (Table 2).

Focal plants—*Flowering phenology*. In 2008, the first plant began flowering on 14 July 2008. The last plants initiated flowering on 31 August 2008, and the last flowers were marked on 6 September 2008, resulting in a flowering season of 54 days. In 2009, the first plant began flowering on 6 July 2009. The last plants initiated flowering on 17 August 2009, and the last flowers were marked on 31 August 2009, resulting in a flowering season of 56 days. The number of flowers marked per plant decreased across the season in 2008 ($X^2_{1,37}=16.39$, $P<0.0001$) and increased across the season in 2009 ($X^2_{1,35}=85.47$, $P<0.0001$) (Figure 2a). Number of flowers marked per day was higher in 2008 versus 2009 at $P<0.07$ (Table 2).

Oviposition. Eggs were observed in both male and female phase flowers. In 2008, the first egg was observed on 19 July, and the last egg was observed on 20 August. In 2009, the first egg was observed on 8 July, and the last egg was observed on 12 August. Oviposition (eggs per flower) decreased across the season in both years (2008:

$X^2_{1,37}$=4.91, *P*=0.0267; 2009: $X^2_{1,35}$=5.21 *P*=0.0224) (Figure 2b). Oviposition was twice as high in 2009 as in 2008, resulting in a difference between years at *P*<0.08 (Table 2). Peak oviposition was over 1.5 times higher in 2009 (2.75 eggs per flower) than in 2008 (1.6 eggs per flower), and peak oviposition was 9 days earlier in 2009 (14 July 2009 versus 22 July 2008).

Larval density. Forty plants (34%) 2008 and 28 plants (29%) in 2009 had at least one larval observation. In both years, 14 plants had two or more dates when a larva was observed. In 2008, the first larva was observed on 24 July, and the last larva was observed on 26 August, with three more flower observations after that date. In 2009, the first larva was observed on 23 July, and the last larva was observed on 25 August, on the last day of flower observations. In both years, peak larval density was 1.5 larvae per plant, and the peak densities occurred on 6 August and 14 August 2008 and 8 August 2009. In 2009, but not 2008, the number of larvae observed per plant increased across the season (2008: $X^2_{1,37}$=1.03, *P*=0.3097; 2009: $X^2_{1,35}$=11.84, *P*=0.0006) (Figure 2). Number of larvae per plant on days across the season did not differ between years (Table 2).

Synchrony and the outcomes of the interaction for host plants—Initiated fruit set decreased across the season in both years (2008: $X^2_{1,37}$=104.83, *P*<0.0001; 2009: $X^2_{1,35}$=13.53, *P*=0.0002). In 2008, predation did not change across the season ($X^2_{1,37}$=0.06, *P*=0.8065), however, in 2009, predation was higher at the end of the season ($X^2_{1,35}$=48.10, *P*<0.0001). Mean initiated fruit set was similar in the two years, but predation was significantly higher in 2009 (Table 2). The mean level of synchrony between host plants and pollinating seed predators was significantly different between

years: approximately two times higher in 2009 (Table 2).

In 2008, two plants had synchrony=0 (no overlap with *H. ectypa* oviposition), and their median flowering date was 31 August with mean flowering duration of less than six days. In 2009, four plants had synchrony=0, and their median flowering dates were 17–24 August with a mean flowering duration of 10 days. These flowering times are beyond the last observed *H. ectypa* egg in both years, and so these plants had no exposure to ovipositing *H. ectypa* moths, thus synchrony=0.

The highest synchrony in 2008 was 0.746, and the plant with this level of synchrony flowered over 6 days with a median flowering date of 21 July (seven days after flowering initiation). The highest synchrony in 2009 was 1.402, and the plant with this level of synchrony flowered over just one marking period on 18 July (all of its flowers were open on this day) (12 days after flowering initiation). These flowering dates are near peak oviposition for both years. See Appendix A for synchrony calculations for eight focal plants.

Host plant synchrony with *H. ectypa* oviposition did not have an effect on initiated fruit set in either year (2008: $X^2_{1,118}=2.33$, $P=0.1271$; 2009: $X^2_{1,96}=2.21$, $P=0.137$) (Figure 3), however, synchrony had a significant effect on predation in both years. In 2008, plants with higher synchrony had higher predation (2008: $X^2_{1,118}=46.47$, $P<0.0001$), but in 2009, plants with higher synchrony had lower predation by *H. ectypa* larvae (2009: $X^2_{1,96}=16.74$, $P<0.0001$) (Figure 3).

Discussion

My results demonstrate a significant role of synchrony in determining plant-pollinating

seed predator interaction outcomes within a season. Although there was no effect of synchrony on initiated fruit set in either year, in 2008, plants with the highest synchrony experienced the highest predation rates, whereas in 2009, plants with the highest synchrony experienced significantly lower predation. This interannual variability in the effect of synchrony on predation may be attributed to between year differences in within season timing of flowering and oviposition as compared to the timing of larval activity. Although pollinator visitation, stigmatic pollen loads and larval density per plant did not vary between years, each of these variables changed significantly across at least one season.

The magnitude of synchrony was significantly different between years and was two times greater in 2009 compared to 2008. Because both flowering and oviposition (the two variables used to calculate synchrony) were delayed to a similar degree in 2008 compared to 2009, these interannual differences likely did not affect synchrony differences between years. Between year differences in 1) within plant flowering patterns and 2) level of oviposition, however, likely were important. Flowering affects synchrony mainly in the way that individual plants open their flowers—do plants open a small or large proportion of their flowers per marking period? In 2009, I found that plants individually flowered over a shorter period, and thus the proportion of flowers opening per marking period was significantly higher in 2009 versus 2008 (Appendix B). With a higher proportion of a plant's flowers open in a marking period, the potential for interaction with *H. ectypa* through oviposition is magnified and will result in a higher synchrony level (in Appendix A, compare marking period 1 for plants T2-9 and P1-136 where oviposition rate is 0.666 for both but proportion of flowers is different resulting in a different synchrony score for

that period on those plants). It is also likely that levels of oviposition were important in determining interannual differences in synchrony. With the lower oviposition levels in 2008, there was a smaller difference in the potential synchrony levels between the most and least synchronous plants, whereas in 2009, plants flowering at peak oviposition had twice the potential synchrony as those flowering after the period of *H. ectypa* oviposition that year.

Synchrony had a significant effect on predation in both years, but in opposite directions. In 2008, plants with the highest synchrony between flowering and oviposition experienced higher predation, whereas in 2009, these plants experienced lower predation. In 2008, the significant positive effect of synchrony on predation indicates that plants with the greatest overlap with *H. ectypa* oviposition suffered the greatest predation levels. The timing between flowering and larval activity could have caused this result. Compared to 2009, later flowering and oviposition but with similar larval phenology as 2009 meant that plants in 2008 with the highest synchrony were less removed from peak larval activity (see more below on larval activity timing and the effect of climate). In 2008, the first flower and first egg were only 10 and 5 days before the first larval observation, respectively. In 2009, however, the first flower and first egg were 17 and 15 days before the first larval observation, respectively. In 2009, the plants with the highest flowering and oviposition overlap (=highest synchrony) had more time to mature their fruits before larval activity peaked, plus fruit maturation was faster in 2009 (Appendix B). Larvae prefer flowers and unhardened fruits (Castillo et al., In preparation), and therefore early flowers in 2009 may have been avoided because they had advanced to mature, hardened fruits by the time that larvae were highly active. In other words,

because of the length of time between oviposition and larval activity in 2009, plants that flower synchronously with oviposition activity may escape predation by the increasingly large larvae with higher consumption rates.

Initiated fruit set and predation showed different within and between season patterns. Initiated fruit set decreased across the season in both years. Although total pollinator density also decreased across the season in both years, stigmatic pollen loads were constant and sufficient to fertilize all ovules (at least two times greater than ovule number = 25, Reynolds et al. 2009) across the season (for all but one sample in 2008), providing no evidence of pollen limitation (Knight et al. 2005). Furthermore, the sufficient stigmatic pollen loads across the season in both years may account for the lack of a relationship between synchrony and initiated fruit set in either year and suggests that the increase in stigmatic pollen loads across 2008 are not biologically significant. If stigmatic pollen loads do not explain the decrease in initiated fruit set across the season, then resource limitation may have been a factor, as in senita cactus for which lower water availability resulted in lower reproduction (Holland 2002). Additionally, ongoing fruit development has been shown to reduce initiated fruit set for later flowers (Fenster 1991c). The pattern of predation across the 2009 season is likely due to a significant increase in larval density across 2009, and the two times higher oviposition in 2009 versus 2008 may be the main cause of differences in predation between years (e.g., Elzinga and Bernasconi 2009). Compared to other studies on the role of flowering phenology on *Silene-Hadena* interaction outcomes, my results were most similar to two studies in particular. Biere and Honders (1996) investigated reproductive success of two *Silene* spp. with different flowering phenologies and found that for the earlier flowering

species (*S. dioica*), early plants had high fruit set and low predation. Collin and Shykoff (2010) also found a decrease in fruit set and an increase in predation across the season. Both results are similar to the patterns for predation that I detected in 2009. The authors did not collect *Hadena* abundance data, and so relative match or mismatch with each species over its flowering duration and the role of synchrony, therefore, could not be determined.

I found interannual differences in the pattern of larval density across the season. What might be most curious about the larval patterns between years is that, unlike flowering and oviposition, larvae first appeared and peaked at nearly the same date in each year and reached a similar peak density. It is possible that abiotic conditions affected larval development rates. Larval development time may be decreased under favorable environmental conditions (Bale et al. 2002), namely higher daytime maximum temperatures during the period when larvae are feeding (Dell et al. 2005), and I found slightly higher maximum daily temperatures during the flowering season in 2008 versus 2009 (Appendix C). Similarly, for two fig wasp species, larval fig wasp maturation was 1.5 times faster when temperatures increased from 24° C to 28° C, and temperature explained over 70 percent of the variation in developmental time (Bronstein and Patel 1992).

Plant flowering phenology and phenology of insect emergence or activity may be affected by climatic variables (Rathcke and Lacey 1985). Studies explicitly focused on the effects of climate change on phenology and synchrony and their effects on interaction outcomes demonstrate that climate change causes mismatches between interacting species (Visser and Both 2005, Thackeray et al. 2010). Temperature, in particular, is

important for plant flowering phenology and insect development (Tauber and Tauber 1981, Bale et al. 2002, Cleland et al. 2007), but the response of plants and insects may be in different directions (van Asch and Visser 2007). Increased temperature resulted in earlier flowering initiation of *Silene acaulis* (Alatalo and Totland 1997). Rainfall may also affect phenology, and for *Ficus* spp., drought caused phenological changes that resulted in local extinction of fig wasps (Harrison 2001). I found earlier flowering and oviposition in 2009, by over one week, when rainfall prior to the flowering season was much greater than in 2008 (540.25 mm in 2009 versus 294.64 mm in 2008, Appendix C). Other climatic variables were less variable between the two years but warrant further investigation in future studies to gain insights on the complexity of interaction outcomes in light of year to year climatic differences (Appendix C).

This is the first study to calculate host plant-pollinating seed predator synchrony for a plant–pollinating seed predator interaction, to my knowledge. I calculated synchrony by combining an individual plant's proportion of flowers open with oviposition rates of the pollinating seed predator. Russell and Louda (2004) used a similar "difference in proportion" metric to explain variation in host plant-seed predator interactions among several populations, while my measure of synchrony is more similar to that calculated by van Asch and Visser (2007) that focused on the bud opening date for individual trees. Like van Asch and Visser (2007), I was interested in the variation in interaction outcomes within a population. These types of results are useful in studying demographic or evolutionary effects of pollinating seed predator interactions within a population (Elzinga et al. 2007b, Miller-Rushing et al. 2010). By calculating synchrony and studying its effects over two years, I incorporated potential effects of extrinsic factors (e.g., climate

effects) on host plant demographic factors, which may subsequently affect the way that mutualism affects population processes (Holland 2002).

Reynolds et al. (2012) demonstrated interannual and geographic variation in visitation rates and oviposition of *H. ectypa* on *S. stellata* and subsequent effects on host plant reproduction. Because of similar pollinator importance to co-pollinators and the additional fruits consumed by *H. ectypa*, Reynolds et al. (2012) concluded that the interaction between *H. ectypa* and *S. stellata* is parasitic, but because of within season variability in pollinating seed predator density, their results suggest that under specific ecological conditions the interaction outcome may be less antagonistic and even potentially mutualistic. This study has now identified that under the very highest levels of synchrony with *H. ectypa*, host plants experience relatively lower flower and fruit predation and at the same time benefit from high fruit initiation rates in one year, while the opposite is true in another year. These patterns, as suggested above, may reflect differences in the greater displacement of larval activity relative to flowering and oviposition in 2009 versus 2008, as well as, potential effects of climate differences between years. This study, therefore, provides an example of a within-season ecological scenario (varying synchrony levels) resulting in interannual differences in interaction outcomes. This insight into the within season and year-to-year variability in the effect of synchrony on host plant reproduction is essential to understanding the ecological forces that shape mutualistic interactions (Billick and Tonkel 2003). Variation in interactions allows populations to respond to changes in their environment (Thompson 1999), thereby helping to explain the persistence of this facultative interaction.

Tables

Table 1. The relationship between number of estimated total visits of *H. ectypa*, moth co-pollinators or all nocturnal moth visits per *Silene stellata* flower with date across the season.

	H. ectypa			Co-pollinators			Total pollinator visits		
	Sign	Chi-square	*P*	Sign	Chi-square	*P*	Sign	Chi-square	*P*
2008	-	87.27	<0.0001	+	6.07	0.0137	-	25.75	<0.0001
2009	-	106.48	<0.0001	+	12.19	0.0005	-	20.81	<0.0001

Table 2. Results of tests comparing means for 2008 and 2009 for nightly number of visits by *H. ectypa*, moth co-pollinators and total moth pollinators; *S. stellata* stigmatic pollen loads; daily number of flowers marked; daily oviposition (eggs observed per flowers marked); daily larvae per plant; initiated fruit set and predation for individual focal plants and synchrony with pollinating seed predator oviposition. Untransformed means and ± 1 standard errors are presented.

				Mean ± S.E.	
Pollinators and pollination	**F**	**df**	***P***	**2008**	**2009**
Pollinator visitation - *H. ectypa*	0.98	42	0.3276	6.56 ± 1.39	4.65 ± 1.32
Moth co-pollinators	1.31	42	0.2595	5.19 ± 0.63	4.23 ± 0.55
Total moth pollinators	2.88	42	0.0971	11.74 ± 1.32	8.88 ± 1.03
Stigmatic pollen load per flower	0.71	544	0.3989	182.66 ± 19.06	184.08 ± 19.62
Focal plants					
Population flower density	3.53	75	0.0644	60.56 ± 8.96	41.24 ± 5.73
Oviposition	3.23	75	0.0765	0.21 ± 0.05	0.44 ± 0.11
Larvae per plant	1.07	77	0.3052	0.71 ± 0.11	0.55 ± 0.10
Initiated fruit set	0.19	74	0.6665	0.75 ± 0.02	0.75 ± 0.03
Predation	6.47	74	0.0131	0.34 ± 0.03	0.46 ± 0.04
Synchrony	27.29	74	<0.0001	0.16 ± 0.01	0.32 ± 0.03

Figures

Figure 1. Number of nightly pollinator visits to *S. stellata* flowers by *H. ectypa*, moth co-pollinators and all nocturnal moth pollinators estimated for the entire moth activity period in A) 2008 and B) 2009. Figures were produced using values obtained from nonparametric regression.

Figure 2. A) Daily number of *S. stellata* flowers marked in 2008 and 2009 and B) daily *H. ectypa* eggs per marked *S. stellata* flower and number of larvae per *S. stellata* plant for 2008 and 2009. For several plant observation dates early in the season, no larvae were observed. The figures were produced using values obtained from nonparametric regression.

Figure 3. The relationship of synchrony between *S. stellata* flowering and *H. ectypa* oviposition with initiated fruit set and flower and fruit predation in A) 2008 and B) 2009. Best fit lines are shown for initiated fruit set (solid line) and flower and fruit predation (dashed line).

Figure 1A.

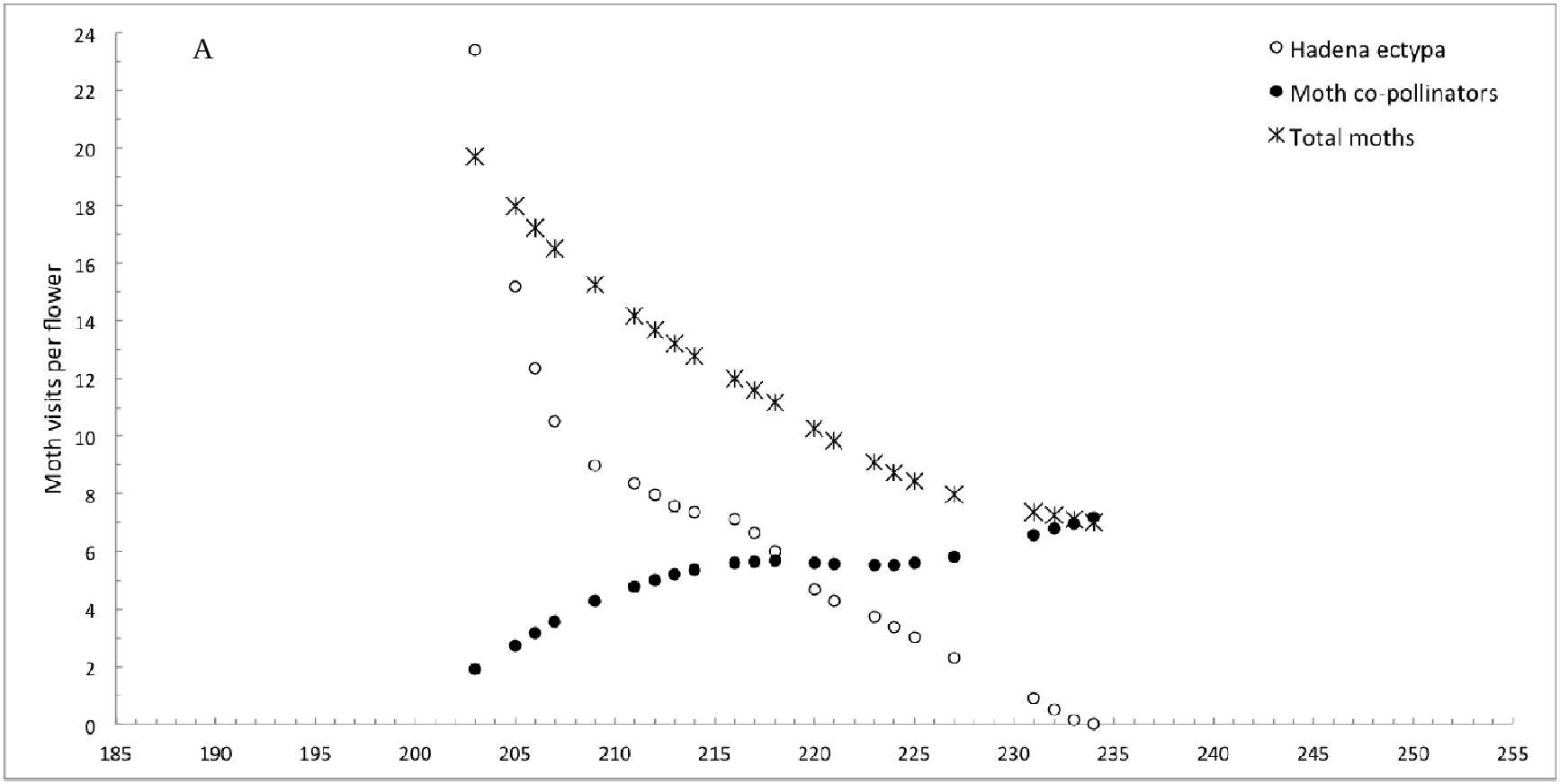

A
Hadena ectypa
Moth co-pollinators
Total moths
Moth visits per flower
0
2
4
6
8
10
12
14
16
18
20
22
24
185
190
195
200
205
210
215
220
225
230
235
240
245
250
255

Figure 1B.

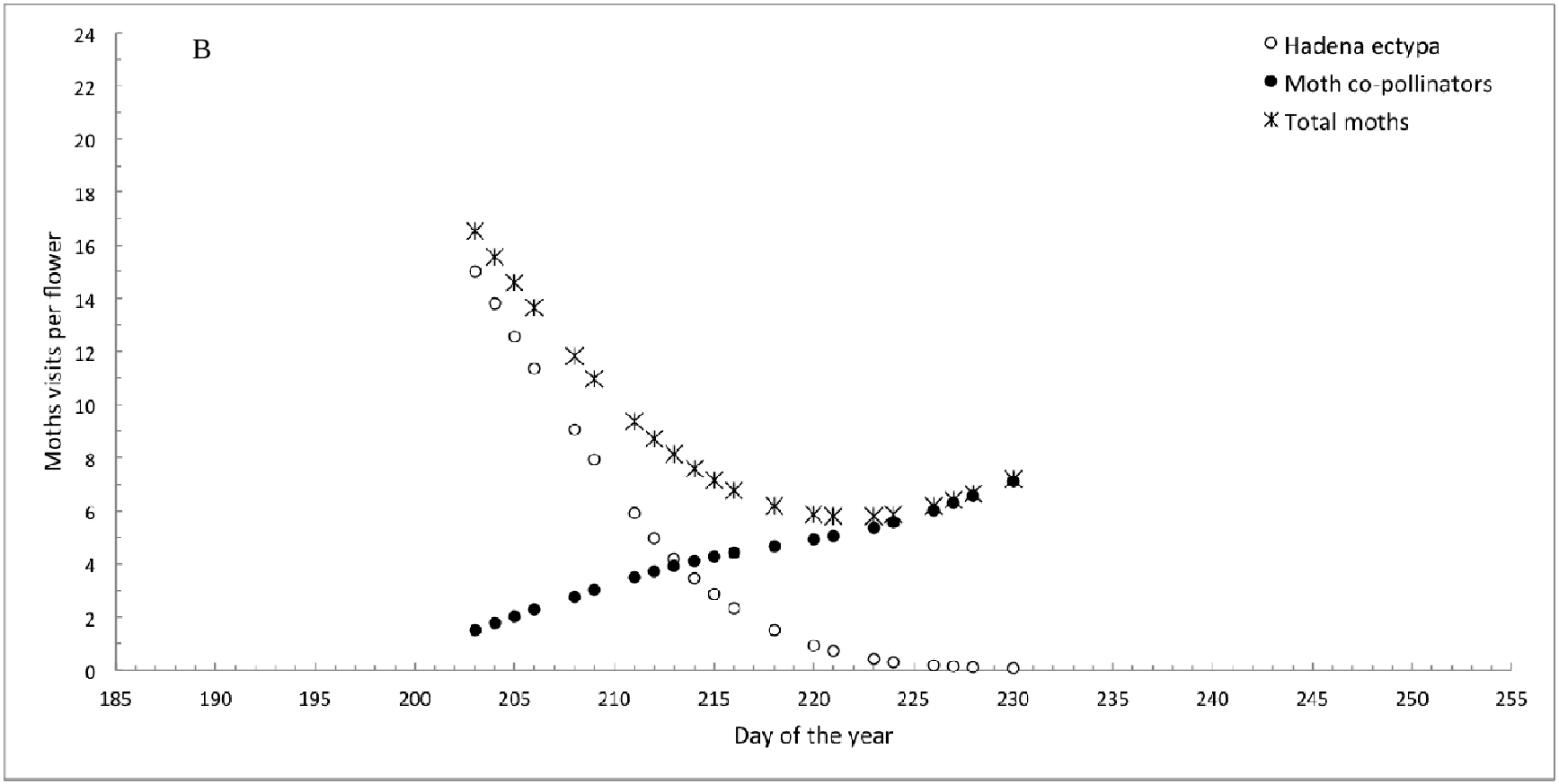

B
Hadena ectypa
Moth co-pollinators
Total moths
Moths visits per flower
Day of the year
0
2
4
6
8
10
12
14
16
18
20
22
24
185
190
195
200
205
210
215
220
225
230
235
240
245
250
255

Figure 2A.

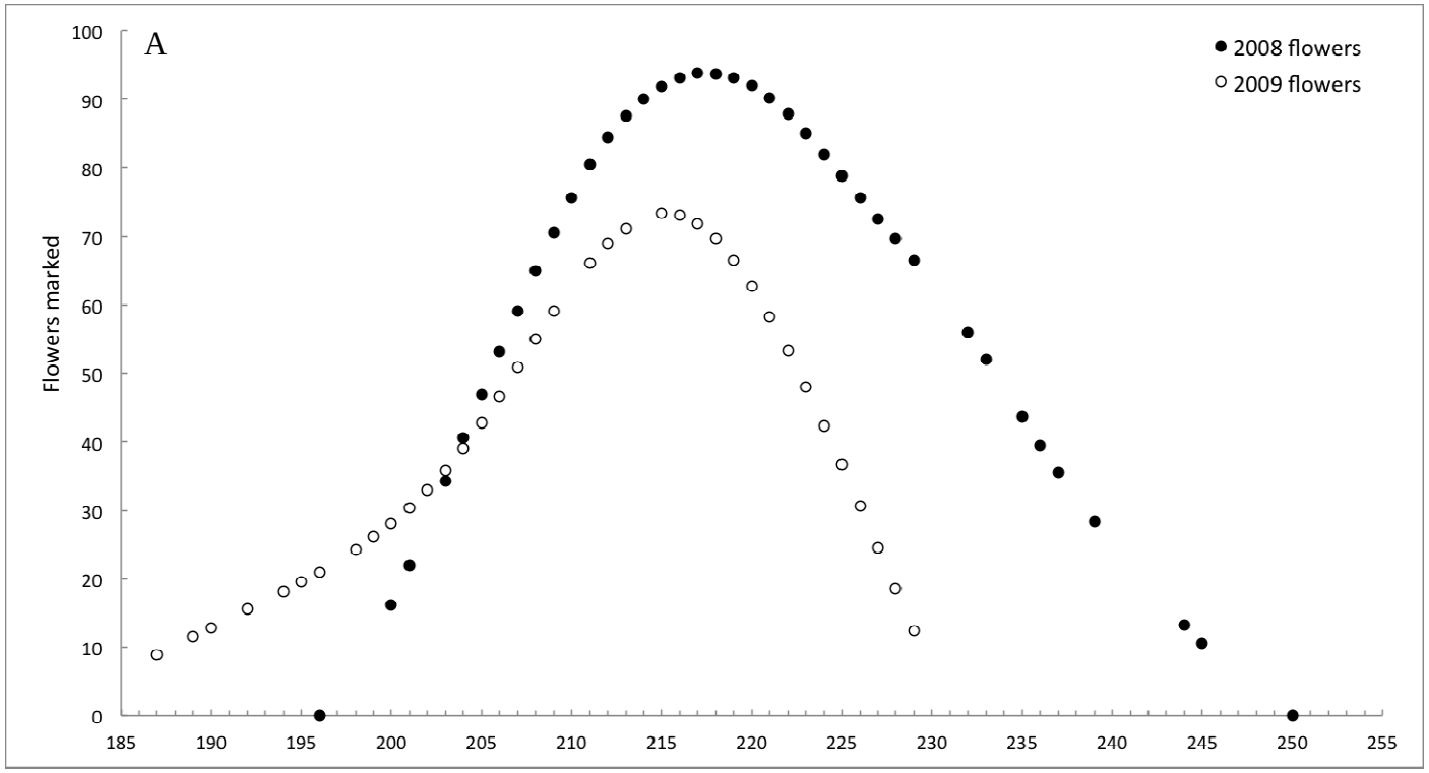

A
2008 flowers
2009 flowers
Flowers marked
0
10
20
30
40
50
60
70
80
90
100
185
190
195
200
205
210
215
220
225
230
235
240
245
250
255

Figure 2B.

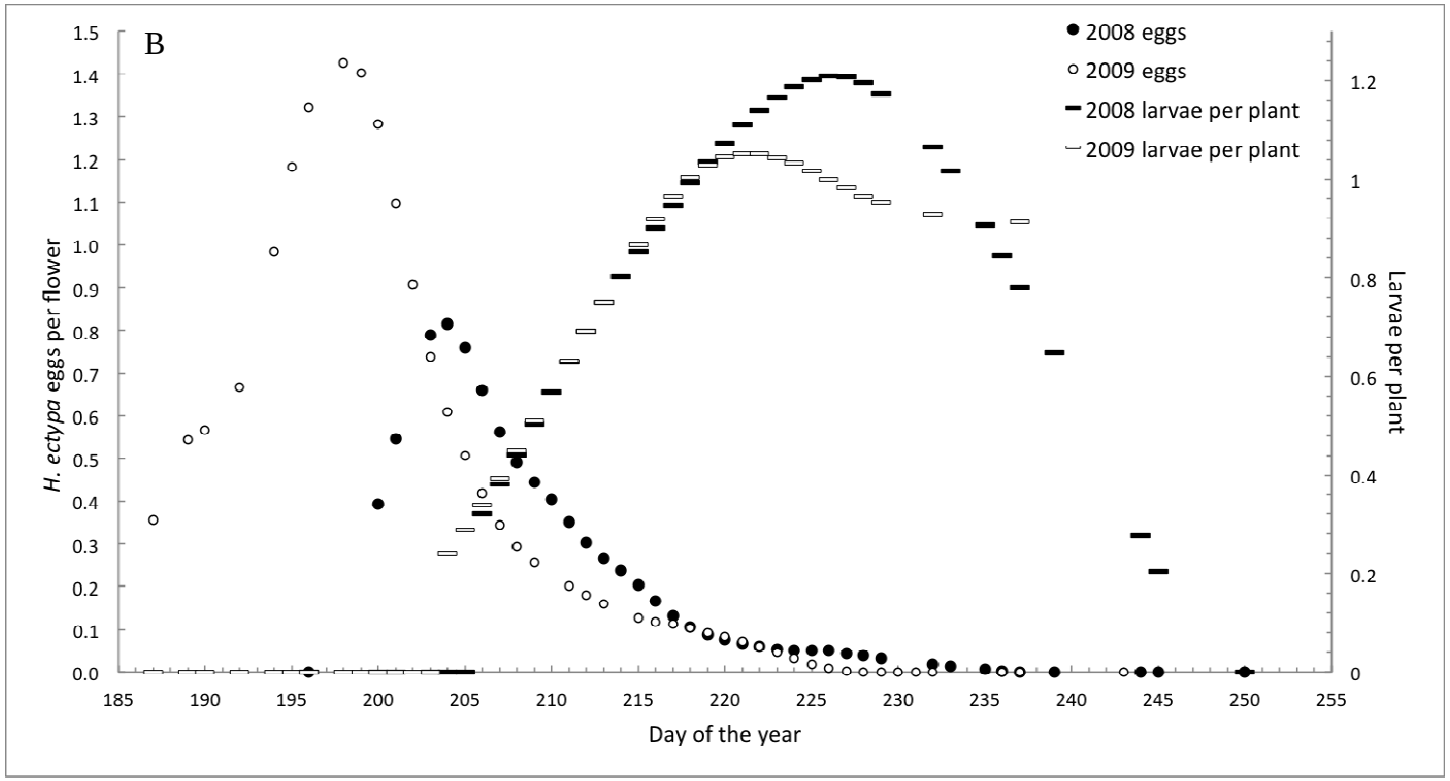

B
2008 eggs
2009 eggs
2008 larvae per plant
2009 larvae per plant
H. ectypa eggs per flower
Larvae per plant
Day of the year
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1.2
1
0.8
0.6
0.4
0.2
0
185
190
195
200
205
210
215
220
225
230
235
240
245
250
255

Figure 3A.

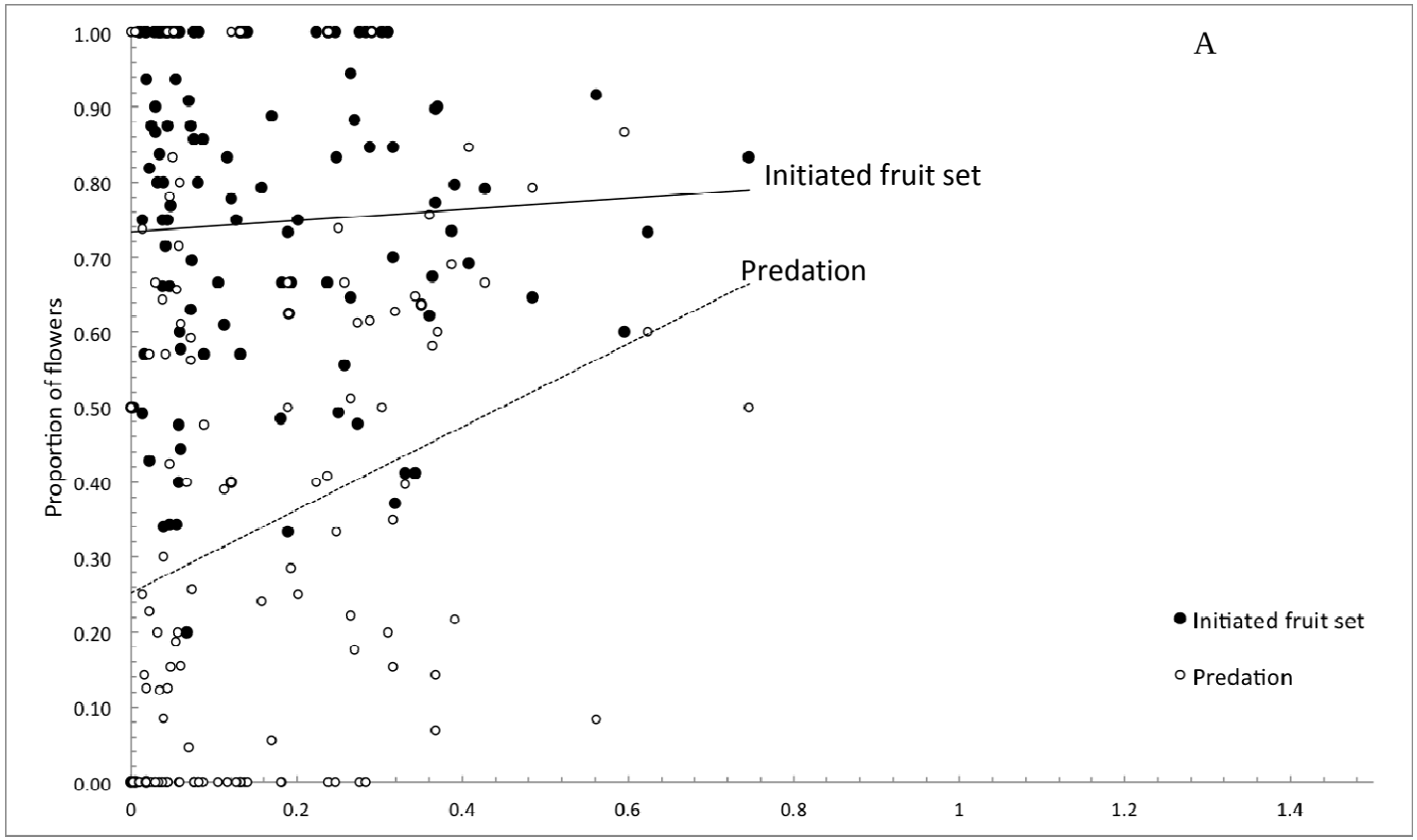
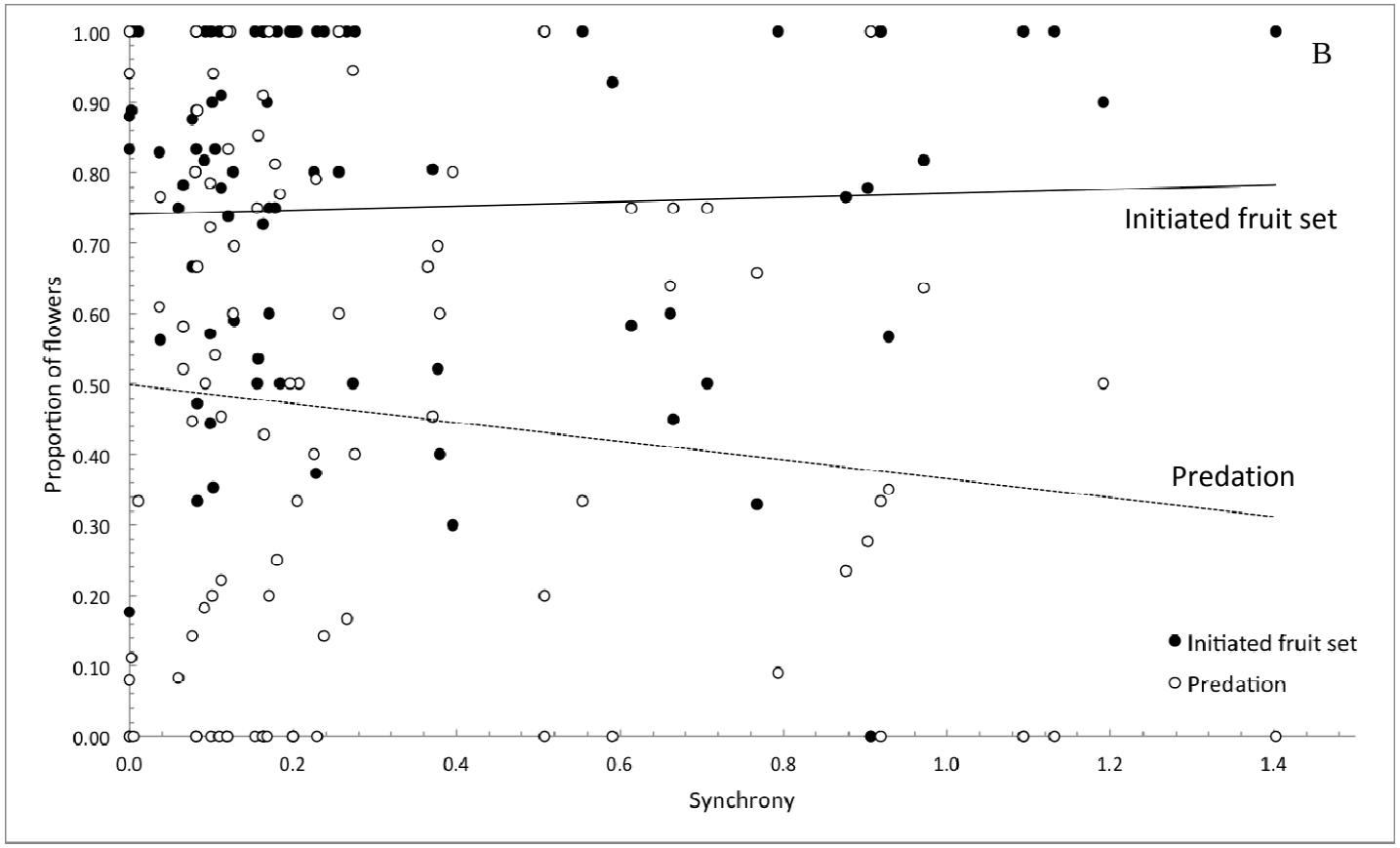

A
Proportion of flowers
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
0.2
0.4
0.6
0.8
1
1.2
1.4
Initiated fruit set
Predation
Initiated fruit set
Predation

Figure 3B.


B
Initiated fruit set
Predation
Initiated fruit set
Predation
Proportion of flowers
Synchrony
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4

Appendices

The following supporting figures and tables provide detailed information to accompany Chapter 3. Appendix A demonstrates the calculations necessary to determine synchrony between *Silene stellata* flowering and *Hadena ectypa* oviposition. Appendix B provides statistical results and means ± 1 standard error of additional flower timing variables and fruit maturation rates. Appendix C is a table of meteorological data collected from Mountain Lake Biological Station in three periods prior to and during *S. stellata* flowering in 2008 and 2009.

Appendix A. Example synchrony calculations for six *Silene stellata* focal plants in 2009 in decreasing order of synchrony. Mark day = date flowers were marked in the field, Numb. fl. = number of flowers marked at that period, Pro. fl. open = proportion of total flowers for that plant that were first open on that marking day, Eggs per fl. = population level estimates of oviposition by *Hadena ectypa* for that date, Synchrony = Pro. fl. open x Eggs per fl. Synchrony values for each focal plant is the sum of synchrony products for each marking date and are in bold. In 2009, the first egg was observed on 8 July, and the last egg was observed on 12 August.

Plant ID	Period	Mark day	Numb. fl.	Pro. fl. open	Eggs per fl.	Synchrony
T2-9	1	11 July	1	0.083	0.6666	0.056
T2-9	2	13 July	3	0.250	0.9839	0.246
T2-9	3	17 July	2	0.167	1.4262	0.238
T2-9	4	20 July	4	0.333	1.0967	0.366
T2-9	5	23 July	1	0.083	0.6082	0.051
T2-9	6	31 July	1	0.083	0.1796	0.015
						0.970
P1-136	1	11 July	4	0.667	0.6666	0.444
P1-136	2	17 July	2	0.333	1.4262	0.475
						0.920
P1-12	1	21 July	15	1.000	0.907	0.907
						0.907
P1-8	1	8 July	4	0.103	0.543	0.056
P1-8	2	11 July	11	0.282	0.667	0.188
P1-8	3	13 July	6	0.154	0.984	0.151
P1-8	4	17 July	13	0.333	1.426	0.475
P1-8	5	27 July	3	0.077	0.294	0.023
P1-8	6	30 July	2	0.051	0.200	0.010
						0.903
P3-21	1	24 July	5	1.000	0.507	0.507
						0.507

T1-207	1	22 July	9	0.081	0.739	0.060
T1-207	2	25 July	8	0.072	0.418	0.030
T1-207	3	28 July	20	0.180	0.256	0.046
T1-207	4	31 July	32	0.288	0.180	0.052
T1-207	5	4 August	24	0.216	0.119	0.026
T1-207	6	7 August	11	0.099	0.092	0.009
T1-207	7	9 August	7	0.063	0.073	0.005
						0.227

P4-139	1	30 July	3	0.600	0.200	0.120
P4-139	2	3 August	2	0.400	0.127	0.051
						0.171

T2-209	1	5 August	2	0.033	0.113	0.004
T2-209	2	7 August	4	0.067	0.092	0.006
T2-209	3	9 August	12	0.200	0.073	0.015
T2-209	4	12 August	18	0.300	0.033	0.010
T2-209	5	14 August	13	0.217	0.009	0.002
T2-209	6	16 August	7	0.117	0.000	0.000
T2-209	7	19 August	4	0.067	0.000	0.000
						0.036

Appendix B. Table of additional interannual differences in additional flowering variables and fruit maturation rates of *Silene stellata*. Inter-annual difference in plant flowering duration, number of flowers (plant size), proportion of flowers open on a marking day and median flowering date were analyzed using a mixed model ANOVA with plant identification as a random factor (Proc MIXED, SAS 9.2). To determine inter-annual differences in fruit maturation rates, I subtracted the marking date of sets of flowers on study plants from mean end-of-the-season collection date of flowers and fruits in that set of marked flowers. I used a mixed model ANOVA with plant identification as a random factor to compare fruit maturation between years (Proc MIXED, SAS 9.2).

				Mean ± S.E.	
	F	df	*P*	2008	2009
Flowering duration per plant	12.61	74	0.0007	10.77 ± 0.71	7.64 ± 0.72
Number of flowers per plant	7.54	74	0.0076	19.38 ± 2.09	15.99 ± 2.03
Prop. flowers open per plant	14.46	580	0.0002	0.28 ± 0.01	0.31 ± 0.01
Median plant flowering date	68.88	74	<0.0001	220.18 ± 0.81	212.44 ± 0.92
Fruit maturation timing (days)	101.47	560	<0.0001	21.3 ± 0.28	16.7 ± 0.40

Appendix C. Meteorological data collected at Mountain Lake Biological Station and available at http://www.mlbs.virginia.edu/mlbsmetdata. Temperature data are in degrees Celsius, humidity is reported in percent and rainfall is in mm. These periods were chosen to examine because they are the periods preceding and during *Silene stellata* flowering at my study site in the years of this study and likely affect flowering timing and *Hadena ectypa* emergence and larval development timing.

	January-March		April-June		Flowering period (3 July-6 Sept.)	
	2008	2009	2008	2009	2008	2009
Max. Temp.	17.5	20.33	28.03	26.29	26.61	25.61
Mean Max. Temp	5.21+0.67	4.52+0.79	17.69+0.64	17.75+0.59	22.24±0.29	21.40+0.30
Min. Temp.	-18.4	-22.28	-3.08	-6.88	7.18	8.08
Mean Min. Temp	-4.32+0.66	-4.81+0.79	8.10+0.57	9.07+0.60	13.51±0.27	13.94+0.28
Overall Mean Temp.	0.51+0.65	-0.16+0.77	12.92+0.58	13.26+0.56	17.69±0.22	17.42+0.24
Max. Humid.	100	100	100	100	100	100
Mean Max. Humid.	95.12+0.88	95.29+0.75	96.52+0.85	96.77+0.81	99.67±0.23	100+0.003
Min. Humid.	14.2	13.3	18	18.7	41.5	47.1
Mean Min. Humid.	56.87+2.41	61.9+2.42	60.11+2.05	64.40+2.32	68.10±1.70	76.00+1.42
Overall Mean Humid.	78.60+1.73	80.93+1.56	80.74+1.48	83.63+1.66	88.11±0.89	92.53+0.64
Total Rain	209.3	236.98	294.64	540.25	217.68	200.91
Numb. Rain Days	37	33	47	43	25	38
Max. Rainfall Day	7.37	8.38	11.94	15.49	12.45	7.11

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