PLANT-MICROBE-ANIMAL INTERACTIONS - ORIGINAL RESEARCH

# Florivore impacts on plant reproductive success and pollinator mortality in an obligate pollination mutualism

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**Abstract** Florivores are present in many pollination systems and can have direct and indirect effects on both plants and pollinators. Although the impact of florivores are commonly examined in facultative pollination mutualisms, their effects on obligate mutualism remain relatively unstudied. Here, we used experimental manipulations and surveys of naturally occurring plants to assess the effect of florivory on the obligate pollination mutualism between yuccas and yucca moths. *Yucca filamentosa* (Agavaceae) is pollinated by the moth *Tegeticula cassandra* (Lepidoptera: Prodoxidae), and the mutualism also attracts two florivores: a generalist, the leaf-footed bug *Leptoglossus phyllopus* (Hemiptera: Coreidae), and a specialist, the beetle *Hymenorus densus* (Coleoptera: Tenebrionidae). Experimental manipulations of leaf-footed bug densities on side branches of *Y. filamentosa* inflorescences demonstrated that feeding causes floral abscission but does not reduce pollen or seed production in the remaining flowers. Similar to the leaf-footed bugs, experimental manipulations of beetle densities within individual flowers demonstrated that beetle feeding also causes floral abscission, but, in addition, the beetles also cause a significant reduction in pollen availability. Path

analyses of phenotypic selection based on surveys of naturally occurring plants revealed temporal variation in the plant traits important to plant fitness and the effects of the florivores on fitness. Leaf-footed bugs negatively impacted fitness when fewer plants were flowering and leaf-footed bug density was high, whereas beetles had a positive effect on fitness when there were many plants flowering and their densities were low. This positive effect was likely due to adult beetles consuming yucca moth eggs while having a negligible effect on floral abscission. Together, the actions of both florivores either augmented the relationship of plant traits and fitness or slightly weakened the relationship. Overall, the results suggest that, although florivores are always present during flowering, the impact of florivores on phenotypic selection in yuccas is strongly mitigated by changes in their densities on plants from year to year. In contrast, both florivores consistently influenced pollinator larval mortality through floral abscission, and *H. densus* beetles additionally via the consumption of pollinator eggs.

**Keywords** Yucca · Florivory · Leptoglossus · Floral abscission · Community context

Communicated by Christina Caruso.

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## Introduction

Pollination mutualisms are an important component of terrestrial communities and strongly impact plant diversity and fitness (Pellmyr 1992). Although pollinators can exert strong selection on plants (e.g., Campbell et al. 1997; Conner 1997; Fenster et al. 2004; Parachnowitsch and Kessler 2010) and have often been assumed to be the main selective agent on floral traits, plants also exist within a larger community of interacting organisms. Plants associate with a suite of other species such as herbivores, seed predators,

nectar robbers, and pathogens, and these other interactions can also contribute to plant evolution. As such, we would expect plant traits to be shaped by the combined effects of these different types of interactions (Brody 1997; Strauss 1997; Pilson 2000; Adler 2008; Caruso et al. 2010). Pollinators will favor suites of traits that enhance pollination services, while at the same time herbivores or nectar robbers may select for traits that deter their attraction. Consequently, the evolution of floral traits may be limited by the additive and non-additive effects of the set of interactions occurring within plant populations (e.g., Herrera et al. 2002; Caruso et al. 2003; Adler and Bronstein 2004; Cariveau et al. 2004; Ornelas et al. 2007; Parachnowitsch and Caruso 2008).

Florivores, in particular, may be more likely to impact plant–pollinator interactions through selection on floral traits because they feed directly on floral tissue. Florivores have direct negative effects on pollination mutualisms by causing loss of floral tissue (Mothershead and Marquis 2000; Cascante-Marin et al. 2009; Oguro and Sakai 2009; Rodriguez-Rodriguez and Valido 2011), a reduction of pollen (Weiss 1996; Krupnick and Weis 1999; McCall 2010), and reduced seed set due to florivore feeding (Louda and Potvin 1995; Krupnick and Weis 1999). In addition, florivores may create indirect negative effects due to changes in nectar production (Irwin and Brody 1998, 2011), flower attractiveness, and inflorescence attractiveness (Leavitt and Robertson 2006; McCall 2008; Cardel and Koptur 2010; Sober et al. 2010) that cause pollinators to reduce time spent foraging within flowers and patches, while, in some cases, pollinators may avoid damaged flowers/inflorescences altogether (Irwin 2000; Leavitt and Robertson 2006; Botto-Mahan et al. 2011). These direct and indirect effects have been shown to constrain the evolution of plant traits associated with pollinator attraction, especially when florivores use the same cues to find flowers as pollinators (Theis 2006; Theis et al. 2007; Kessler et al. 2008; Galen et al. 2011; Lay et al. 2011; Theis and Adler 2011).

Given that florivores are a common component of plant–pollinator communities, they have the potential to influence the ecological and evolutionary dynamics of many plant–pollinator interactions (McCall and Irwin 2006). Although the impact of florivores has been studied widely in a number of facultative mutualisms, their effects on obligate mutualism remain relatively unstudied. This is somewhat surprising as obligate mutualisms are used frequently in theoretical treatments of mutualism because these interactions are simple, often involving only a pair of mutualist species. From this perspective, obligate mutualisms are an excellent starting point for examining the influence of florivores and other antagonists on mutualism, and a strong theoretical framework in which to place these additional interactors already exists (e.g., Bronstein et al. 2003; Morris et al. 2003; Jones et al. 2009).

The pollinating seed–parasite mutualism between yuccas and yucca moths is a classic example of obligate mutualism (Powell 1992; Pellmyr 2003). Yuccas require yucca moths for pollination and the moth larvae feed exclusively on yucca seeds. In a correlative study examining the influence of florivores on this mutualism, Althoff et al. (2005) used a combination of flowering traits, insect surveys, and structural equation modeling to examine the effects of two florivore species and the yucca moth pollinator *Tegeticula cassandra* (Lepidoptera: Prodoxidae) on fruit set in *Yucca filamentosa* (Agavaceae). The effect of florivores on fruit set yielded mixed results: the generalist florivore *Leptoglossus phyllopus* (Hemiptera: Coreidae), the leaf-footed bug, decreased fruit production, whereas the specialist beetle *Hymenorus densus* (Coleoptera: Tenebrionidae) had no effect. Both florivores' peak abundance coincided with peak plant flowering; however, peak pollinator moth abundance occurred earlier than peak flowering. Althoff et al. (2005) suggested that, because pollinator larvae develop within flowers and fruits, pollinators may be under selection to emerge early in order to avoid increased larval mortality caused by florivory, primarily through floral abscission. Later, Segraves (2008) demonstrated that the beetle *H. densus* consumes pollinator moth eggs while feeding on style tissue in *Y. filamentosa* flowers.

In this study, we used two approaches to examine the direct effects of the leaf-footed bugs and beetles on plants and moth pollinators. First, we examined the mechanistic basis by which each of these florivore species may affect seed set and pollen availability in *Y. filamentosa*. Specifically, we used independent experimental manipulations of adult leaf-footed bug and beetle densities on naturally occurring plants to test if feeding increased floral abscission and decreased pollen availability. Because leaf-footed bugs feed on xylem and phloem in buds, flowers, and fruit, we additionally tested whether this florivore reduced seed set in retained flowers. Second, we performed daily surveys of the florivores and measurements of flowering traits and seed set from flowering periods in each of 3 years. Based on these surveys, we used path analysis to elucidate the pattern of phenotypic selection on flowering traits and the role of florivores in influencing this pattern. We also examined the effect of florivores on the pollinator larval loads within *Y. filamentosa* fruit.

## Materials and methods

### Study species

*Yucca filamentosa* (Agavaceae) is a native perennial growing in disturbed areas throughout the southeastern United States. Plants used in the study were located at the

Archbold Biological Station (27°10′48″N, 81°19′48″W) and in a patchwork of undeveloped housing lots in Placid Lakes, Florida (27°15′22″N, 81°22′57″W) near the field station. Individuals grow as a basal rosette of leaves and typically flower every 2–3 years. Flowering at the study site occurs April through June. A *Y. filamentosa* inflorescence ranges between 0.5 and 4 m tall, can have 20 or more side branches, and 300 or more flowers that open over the course of 1–3 weeks. Flowers open at dusk and remain open until the following morning. Flowers not actively pollinated by the yucca moth *Tegeticula cassandra* during the first night are abscised from the plant. The capsular fruit mature in approximately 2 months, dehisce, and the seeds disperse via wind.

Two florivores, the leaf-footed bug *L. phyllopus* and the beetle *H. densus*, feed on *Y. filamentosa* inflorescences. The host range of the leaf-footed bug includes many plant families and this species is also a pest on many agricultural plants (Allen 1969; Baranowski and Slater 1986). A single *Y. filamentosa* inflorescence can host 1–44 feeding adults per day (Althoff and Segraves, personal observation). The tall *Y. filamentosa* inflorescence may serve as a convenient location for adults to congregate as we have observed adults both feeding and mating on inflorescences. Females will also lay eggs on plants and the aposematically colored nymphs also feed on the inflorescence. An individual uses its stylets to pierce into the buds, flowers, and fruit to feed on plant fluids. In contrast, the beetle *H. densus* appears to be a yucca specialist and was first noted on yuccas by Brues (1926). Dozens of beetles have been observed within single flowers, and individual plants can have more than 500 beetles. Adults congregate and mate within flowers and use their chewing mouthparts to feed on pollen and style tissue. Segraves (2008) demonstrated that beetles feeding on style tissue also inadvertently eat pollinator moth eggs that are laid in the style.

### Experimental manipulations of florivores to examine effects on plant reproductive success

*Leptoglossus phyllopus experiment*. In April 2008, side branches of 13 *Y. filamentosa* individuals located at the Archbold Biological Station were randomly assigned to a treatment containing 0, 1, 2, or 4 leaf-footed bug adults. Each plant had all treatments. These treatments were chosen based on the range of observed densities of individuals on side branches in 2001 and 2002 (Althoff et al. 2005). Side branches were chosen that had at least six buds. The treatment was initiated on a side branch when the first flower on the side branch opened. A sleeve cage made from vinyl screening sewn around wire hoops was then placed over the next five consecutive buds. The side branch was randomly chosen to be a control or receive one of the

treatments, and the appropriate number of adult leaf-footed bugs was added to the cage. If an adult died, it was replaced with a new individual. Cages were checked daily for 7 days and any buds or flowers that were abscised were recorded. Flowers that opened had one anther removed for pollen grain counts, were hand-pollinated with excess outcrossed pollen to ensure there was no pollen limitation, and given a tag with the pollination date. The entire inflorescence was enclosed by a frame covered with a mesh cage to prevent any additional naturally occurring leaf-footed bugs or other insects from feeding on the inflorescence through the vinyl sleeve cages. After 7 days, the leaf-footed bugs were removed and the side branch cages and inflorescence cage were left on the plant until the seeds were mature. The fruit were collected and the total numbers of viable and inviable seeds were counted. Seed viability is readily assessed based on color: black seeds are viable and yellow or white seeds are inviable. Pollen and anthers were stained with basic fuschin dye and the pollen grains were manually counted using a Leica stereomicroscope at ×40 magnification. Due to the intensiveness of pollen grain counting, only pollen from the controls (0 leaf-footed bugs) and the highest treatment density (4 leaf-footed bugs) were counted.

*Hymenorus densus experiment*. In April of 2006, individual flowers from nine plants were randomly assigned to receive a treatment of 0, 2, 4, or 16 beetles added to individual flower bags. The appropriate number of beetles was placed in a fine mesh bag, the flower was hand-pollinated with outcross pollen, and the bag was placed over the flower and closed with a wire tie around the petiole. These numbers were chosen based on previous surveys of beetle densities (Althoff et al. 2005). After 24 h, the bag and beetles were removed, the number of anthers with pollen were recorded, and any remaining pollen was collected and placed in pre-weighed tin capsules to determine pollen weight. Yucca pollen is extremely sticky which made pollen collection relatively easy and complete. Bags with beetles were replaced on the flower and left for another 48 h. Seventy-two hours is the period over which flowers will be abscised or retained on the plant. Flowers were checked daily to determine if they had abscised.

### Statistical analysis

Data were checked for normality and equal variances before conducting statistical analyses. For the manipulative experiment involving the addition of leaf-footed bugs to side branches, the number of flowers abscised did not conform to normality or equal variances, nor did any transformations of this variable. Thus, the effect of leaf-footed bugs on floral abscission was analyzed via logistic regression with the number flowers abscised treated as an ordinal variable and plant individual used as a blocking factor.

The number of pollen grains was square root-transformed and the effect of leaf-footed bugs on pollen production was analyzed via ANOVA with plant as a blocking factor. An ANOVA with plant as a blocking factor was also used to examine the effect of leaf-footed bugs on seed production.

For the beetle experiment, differences in floral abscission among treatments were tested using nominal logistic regression with plant individual as a blocking factor. The effect of beetles on pollen retention was analyzed in two ways. First, we used logistic regression to assess the effect of beetle density on the number of anthers retaining pollen. The number of anthers was treated as an ordinal variable and plant individual was used as a blocking factor. Second, we used ANOVA with plant as a blocking factor to test for differences in the relative amount of pollen remaining by comparing the total weight of remaining pollen.

Surveys of naturally occurring florivores and pollinator larvae on Yucca filamentosa

In 2008, 2009, and 2010, daily surveys were conducted on the majority of flowering plants in a 533-ha area in Placid Lakes, FL. For each plant, the total number of *L. phyllopus*, *H. densus*, and the number of open flowers were counted. We also measured the following plant characteristics known to influence pollinator visitation and seed set: inflorescence height (cm), date of first flower, duration of flowering (days), and average petal length (mm) of three flowers (Althoff et al. 2005). All fruit were collected from each plant and the number of viable seeds and the number of pollinator larvae was determined for each fruit.

Data collected from the daily surveys of naturally occurring plants were analyzed using path analysis to elucidate the pattern of phenotypic selection on flowering traits (Kingsolver and Schemske 1991; Scheiner et al. 2000). The number of viable seeds produced per plant was used as a measure of plant fitness. The path diagram is a hypothesis of the causal links among variables and their relationship to fitness. Based on Althoff et al. (2005) and the results of the experimental manipulations of florivore densities, we assumed that the path diagram was the actual relationship (Fig. 1). Plant flowering traits have a direct role in determining relative fitness and also in attracting the two florivores that have a direct effect through their feeding on plant relative fitness. Thus, plant traits have two ways to influence fitness: directly and indirectly, via the path through each florivore species. The total amount of direct selection is the combined effects of each path from a plant trait to relative fitness. Florivore abundance was measured as the natural log of the total number of each florivore observed during the flowering period of a plant. Relative fitness was determined by dividing the seed set for each plant by the seed set value for the plant with the highest seed set produced in

a season. Plant traits and florivore abundances were standardized before being used in the path analysis. Because the standardized florivore variables were not normally distributed and no data transformation would correct this problem, we tested the significance of these variables using generalized linear models with a Poisson distribution, and a log link function (Colautti and Barrett 2010). All statistical analyses were performed using JMP 9.0.2 (SAS, Cary, NC, USA).

## Results

Experimental manipulations of florivore densities

The effect of leaf-footed bugs on floral abscission was dependent on the number of individuals feeding within a cage (Table 1a). The mode of flowers abscised increased significantly over control levels with 2 and 4 leaf-footed bugs. In cages with 4 leaf-footed bugs the mode of flowers abscised was five, which represents all of the flowers in a cage. In contrast, neither the number of pollen grains produced in surviving flowers nor the number of viable seeds differed across leaf-footed bug treatments. Individual variation among plants also significantly influenced floral abscission, the number of pollen grains, and the number of seeds (Table 1a).
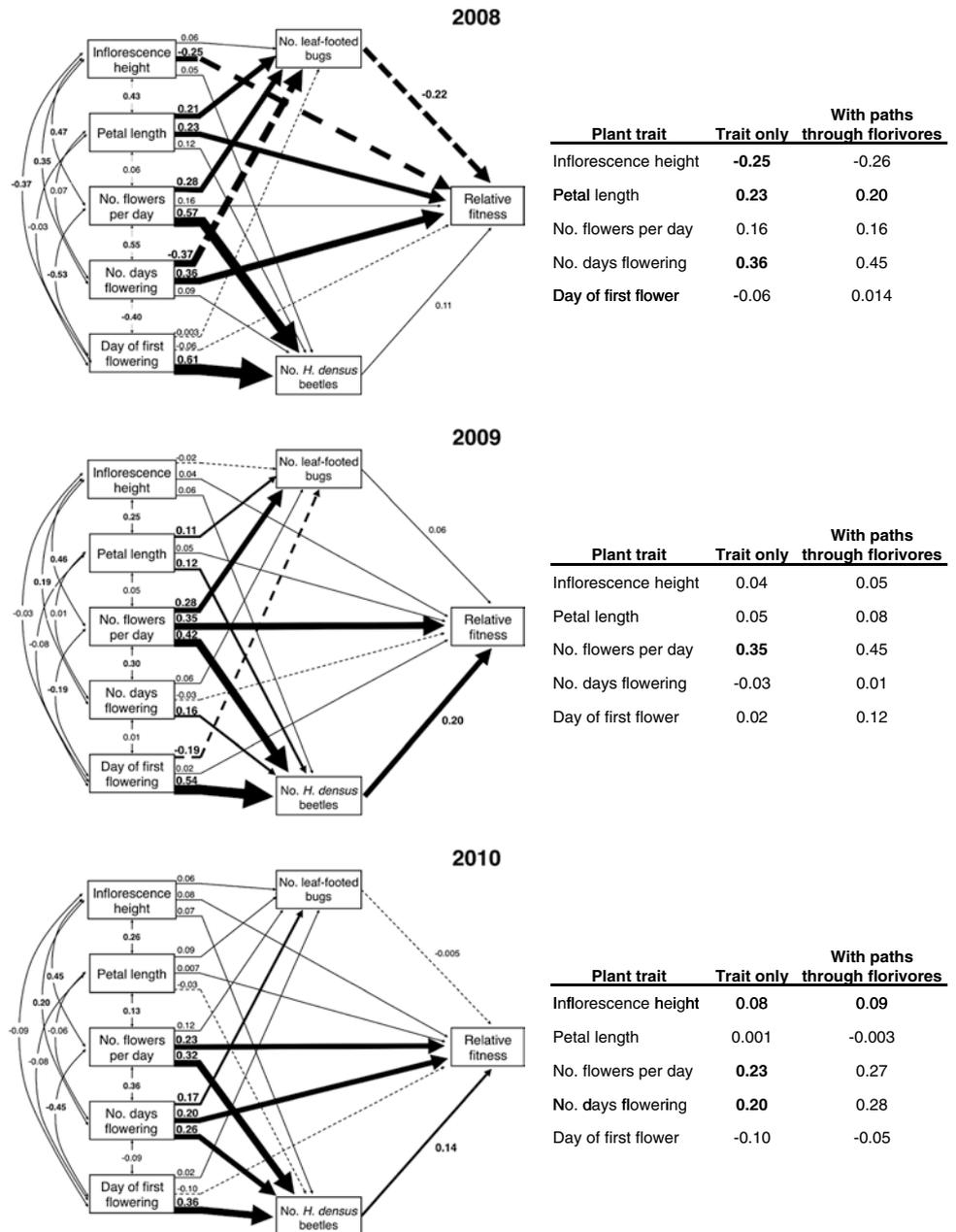
Similar to leaf-footed bugs, the effect of beetles on floral abscission and pollen removal were also dependent on the number of individuals within cages (Table 1b). The proportion of flowers abscised significantly increased with increasing numbers of beetles and the mode of anthers with pollen significantly decreased. The average weight of remaining pollen was also significantly reduced with increasing beetle density in the flower bags. Individual plant responses contributed to differences in floral abscission and the average weight of remaining pollen (Table 1b).

Surveys of a natural population of Yucca filamentosa

The number of flowering plants and florivores varied widely from year to year (Table 2). There were four-fold differences in the number of flowering plants and nine-fold differences in the number of *H. densus* beetles counted. Leaf-footed bugs remained relatively stable across years. The differences in numbers of flowering plants and florivores resulted in differences in the distribution of florivores across plants and flowers. In 2008, the average number of leaf-footed bugs counted per plant and the average number of beetles per flower were three times and six times higher than in the subsequent 2 years.

Path analyses demonstrated that selection on plant traits and the effects of florivores on plant relative fitness was a complicated pattern that varied across years (Fig. 1). Leaf-footed bugs had a significant negative effect on relative

**Fig. 1** Path diagrams used to analyze phenotypic selection on plant flowering traits in *Yucca filamentosa* for 3 years (2008–2011). Relative fitness was based on number of seeds produced per individual plant. Numbers in *bold* represent significant path coefficients or correlations among flowering traits ($P < 0.05$). Width of the path *arrows* illustrates the magnitude of the path coefficients. *Tables* give magnitude of selection gradients with and without the inclusion of the effects due to two florivores, the leaf-footed bug *Leptoglossus phyllopus* and the beetle *Hymenorus densus*



| Plant trait | Trait only | With paths through florivores |
|---|---|---|
| Inflorescence height | **-0.25** | -0.26 |
| **Petal** length | **0.23** | **0.20** |
| No. flowers per day | 0.16 | 0.16 |
| No. days flowering | **0.36** | 0.45 |
| **Day of first flower** | -0.06 | 0.014 |

| Plant trait | Trait only | With paths through florivores |
|---|---|---|
| Inflorescence height | 0.04 | 0.05 |
| Petal length | 0.05 | 0.08 |
| No. flowers per day | **0.35** | 0.45 |
| No. days flowering | -0.03 | 0.01 |
| Day of first flower | 0.02 | 0.12 |

| Plant trait | Trait only | With paths through florivores |
|---|---|---|
| **Inflorescence height** | **0.08** | **0.09** |
| Petal length | 0.001 | -0.003 |
| No. flowers per day | **0.23** | 0.27 |
| **No. days flowering** | **0.20** | 0.28 |
| Day of first flower | -0.10 | -0.05 |

fitness in 2008 but no effect in the other 2 years. Beetles had a significant positive effect on plant fitness in 2009 and 2010 but no significant effect in 2008. The importance of plant traits in determining florivore abundances also differed from year to year. The one exception was that the date of first flower had a large significant positive effect on beetle abundance in every year. Plants that flowered earlier had fewer beetles. For the five flowering traits measured, none had a consistent significant effect on plant fitness across years as represented by the paths from plant traits directly to relative fitness. Two traits, the number of flowers per day and the number of days flowering, were positive and significant in 2 of 3 years.

In terms of overall selection on plant traits, all the traits measured had an effect on plant fitness both directly and through their relationships with florivore abundances. Examination of the selection gradients demonstrates that the florivores influenced the magnitude of overall selection on plant traits (Fig. 1). For example, in 2008, leaf-footed bugs had a negative effect on relative fitness and were negatively related to the number of days that plants flowered. As a result, leaf-footed bugs strengthened selection to increase the number of days flowering. In other instances, for example, petal length in 2008, the effect of florivores was counter to directional selection on plant traits, which reduced the overall selection gradient for this trait. Even so,

**Table 1** Effects of experimental manipulation of (a) *Leptoglossus phyllopus* leaf-footed bugs and (b) *Hymenorus densus* beetles numbers on *Yucca filamentosa* reproductive traits

| Variable | Number of *L. phyllopus* leaf-footed bugs | | | | Treatment effect | Plant effect |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 4 | | |
| (a) *Leptoglossus phyllopus* leaf-footed bugs | | | | | | |
| No. flowers abscised (mode) | 0/5 | 1/5 | 4/5 | 5/5 | $X^2 = 49.39$*** | $X^2 = 41.68$*** |
| No. pollen grains (mean ± SE) | 3,453 ± 155 | – | – | 3,214 ± 140 | $F = 0.71^{NS}$ | $F = 4.59$** |
| No. viable seeds (mean ± SE) | 129 ± 9 | 110 ± 9 | 96 ± 9 | 106 ± 14 | $F = 1.99^{NS}$ | $F = 7.75$** |

| Variable | Number of *H. densus* beetles | | | | Treatment effect | Plant effect |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 16 | | |
| (b) *Hymenorus densus* beetles | | | | | | |
| Proportion flowers abscised | 0.23 | 0.34 | 0.30 | 0.52 | $X^2 = 40.71$*** | $X^2 = 293.62$*** |
| Anthers with pollen (mode) | 6/6 | 0/6 | 0/6 | 0/6 | $X^2 = 976.32$*** | $X^2 = 4.13$ |
| Pollen weight (mg) (mean ± SE) | 0.66 ± 0.04 | 0.24 ± 0.03 | 0.10 ± 0.02 | 0.01 ± 0.001 | $F = 117.54$*** | $F = 9.12$** |

Treatment effect tests for effects of the experimental manipulation of insect numbers and plant effect tests for the effect of individual plants on which the manipulations were randomized

*NS* non-significant

** $P < 0.01$, *** $P < 0.001$

there were no instances in which florivore abundances on plants changed the direction of selection for a plant trait.

In addition to influencing plant fitness, florivores also had an effect on pollinator larval survivorship. The increase in floral abscission by leaf-footed bugs resulted in fewer larvae per plant in 2008 (regression coefficient, −0.70, $P < 0.004$), but not in 2009 or 2010. Beetles had two possible effects on pollinator moth larvae. First, increased abscission could result in loss of pollinator moth larvae; however, there was no effect of the total number of beetles per plant on fruit set in any year (data not shown). Second, beetle feeding within flowers could reduce the number of moth larvae per fruit as a result of consumption of moth eggs. In 2008 and 2009, the average number of beetles in a flower resulted in fewer moth larvae per fruit (2008 regression estimate, −0.16 ± 0.08, $P < 0.007$; 2008 regression estimate, −0.08 ± 0.03, $P < 0.002$, generalized linear model with poisson distribution and log link function). In 2010, there was no effect, and this was also the year with the lowest average number of beetles per flower.

## Discussion

Historically, many evolutionary and ecological studies of pollination mutualisms focus on the interaction of pollinators and plants in isolation of other community members (Bronstein and Barbosa 2002; Stanton 2003; Strauss and Irwin 2004). Other community members, however, can exert strong selection on traits that are important to plant–pollinator interactions (Gomez 2003; Irwin et al. 2004; Ashman and Penet 2007; Teixido et al. 2011). Florivores, in particular, have been shown to exhibit strong effects on many plant traits. For example, Theis and Adler (2011) used experimental manipulations of floral scent to demonstrate that florivores may constrain the evolution of floral scent. Plants with increased levels of fragrance attracted more florivores and had lower seed set. Kawagoe and Kudoh (2010) demonstrated that florivory selected for earlier flowering in *Arabidopsis halleri*. A comparison of two populations with and without high levels of florivory showed increased fitness for early flowering in the population with florivores present. Florivores may also select for changes in the ratio of male and female flowers. Wise and Hebert (2010) demonstrated that florivores of the horse nettle *Solanum carolinense* generated directional selection against production of male flowers. Such male-biased florivory has lead to the evolution of defensive compounds and structures (Tsuji and Sota 2010), and has influenced macroevolutionary patterns of plant defense (Armbruster et al. 2009; Hanley et al. 2009). Consequently, the evolution of traits involved in pollination mutualism will likely

**Table 2** Flowering characteristics of *Y. filamentosa* and florivore abundances of *Leptoglossus phyllopus* leaf-footed bugs and *Hymenorus densus* beetles near the Archbold Biological Station, Lake Placid, Florida

| Observation | Years | | |
|---|---|---|---|
| | 2008 | 2009 | 2010 |
| Number of plants flowering | 120 | 482 | 367 |
| Date of first flower (day of the year) | 104 | 105 | 124 |
| Number of leaf-footed bugs counted | 2,214 | 2,594 | 2,062 |
| Number of *H. densus* beetles counted | 80,605 | 74,262 | 9,121 |
| Number (mean ± SE) leaf-footed bugs per plant | 18.11 ± 2.46 | 5.13 ± 0.52 | 5.51 ± 0.47 |
| Number (mean ± SE) beetles per flower | 7.09 ± 0.68 | 1.33 ± 0.12 | 0.27 ± 0.03 |

stem from the collection of selective pressures by mutualists and antagonists.

In this study, we demonstrate that the effects of florivores on plant reproductive success and pollinator larval mortality may have important consequences for the pollination mutualism between *Y. filamentosa* and its obligate pollinator moth *T. cassandra*. Previously, Althoff et al. (2005) used path analysis to demonstrate that leaf-footed bugs had a negative effect on fruit set, whereas beetles had no effect. Here, we used experimental manipulations of florivore numbers and examined the consequences on seed production rather than fruit production. Specifically, we were able to examine if florivore effects on seed set are manifested through floral abscission and the shunting of plant resources away from reproductive structures. Feeding by leaf-footed bugs significantly increased floral abscission, but did not influence pollen availability or seed set in retained flowers and fruit (Table 2). These results suggest that the energetic costs of leaf-footed bug feeding do not appear to be substantial enough to change nutrient allocation patterns to pollen or seeds developing within *Y. filamentosa* flowers. The major negative effect of leaf-footed bugs is to increase floral abscission, which is likely caused by increased damage to floral tissue.

Similar to leaf-footed bugs, the beetle *H. densus* also caused floral abscission, particularly when beetle densities were high. In addition, beetles had a significant negative impact on the amount of pollen that was retained in mature flowers of *Y. filamentosa*. Just a few beetles within a flower can consume all the pollen within that flower. Adults begin by feeding on pollen residing on anthers and then move to the pollen-packed stigma (Althoff and Segraves, personal observation) This attraction to pollen likely leads to the consumption of moth eggs as beetles continue feeding from the stigma down the length of the style. At high beetle densities within flowers, pollen on anthers is quickly depleted and severe damage to the style results (Segraves 2008).

The surveys of florivore abundance on flowering plants across 3 years demonstrated that the effect of florivores on both plants and pollinator moth larvae shifted from year to year as the number of flowering plants and the timing of flowering changed (Table 1). In 2008, there were relatively few plants flowering compared to 2009 and 2010. As a consequence, florivore densities were high. This pattern changed in 2009 and 2010 because there were three times as many plants flowering. In 2010, the delayed flowering of the entire population of *Y. filamentosa* also served to change florivore abundances, particularly for the beetles. Together, these changes created year-to-year variation in the significant effects of both florivores on both the plants and pollinator moth survivorship.

In at least 1 year, each of the florivores had a significant negative effect on the survivorship of pollinator moth larvae within flowers and fruit. Floral abscission caused by leaf-footed bugs resulted in significantly fewer larvae per plant in 2008, but not in the other 2 years. The small number of plants flowering in 2008 resulted in high leaf-footed bug density. This increased density substantially increased floral abscission and, as a result, mortality of pollinator larvae within abscised flowers. In the other 2 years, densities of leaf-footed bugs were low enough that their effect on abscission and moth mortality was not detectable over the baseline abscission rate in *Y. filamentosa*. Yuccas typically exhibit floral abscission rates as high as 90 % even when controlling for pollen quality and quantity (Althoff et al. 2004). Feeding by *H. densus* beetles decreased moth larval survivorship through increased floral abscission and direct consumption of moth eggs. In 2008 and 2009, the average number of moth larvae per fruit significantly decreased as the average number of beetles per flower increased. In 2010, the late flowering of the plants resulted in an average of less that one beetle per flower. Based on the beetle caging experiments, this density was too low to cause floral abscission or consumption of moth eggs. Overall, florivore feeding, either directly on moth eggs or indirectly on flowers, appears to cause pollinator moth survivorship to decrease.

The effects of the two florivores on seed set and relative fitness among individuals of *Y. filamentosa* is a complicated pattern that highlights the differences between the two florivores in terms of their actions on pollinator larval survivorship. As demonstrated by the experimental manipulations, both florivores cause floral abscission at high densities that could reduce overall fruit set and indirectly kill pollinator

moth larvae in abscised flowers. Thus, leaf-footed bugs and beetles would have similar effects on plant fitness and pollinator moth survivorship. At lower densities, however, florivore feeding may not cause enough damage to induce floral abscission. In this case, the beetles may actually increase plant fitness by feeding on moth eggs. Florivory by the beetles, then, would have a positive effect on seed set in retained flowers.

The path analyses further demonstrated the variable effects of the florivores and flowering traits on relative fitness in *Y. filamentosa* (Fig. 1). There were no consistent effects of florivores or plant traits on relative fitness across the 3 years. For 2009 and 2010, however, two traits, the number of days flowering and the date of first flower, positively influenced relative fitness directly and indirectly via the number of *H. densus* beetles on a plant. Increased flowering duration and flowering later in the season resulted in increased beetle numbers on a plant, which in turn increased relative fitness. The path analyses also indicated that the effects of plant traits on florivores did little to change the direction of selection on plant traits. In no case were standardized regression coefficients for the paths from plant traits to florivores to relative fitness large enough to offset the strength of selection via the direct paths from plant traits to relative fitness. In many cases, the effects of florivores served to augment selection. For example, in 2009, including selection on the number of flowers per day via paths to the florivores changed the selection gradient from 0.35 to 0.45.

The variability in phenotypic selection on *Y. filamentosa* flowering traits from year to year can best be explained by changes in the number of flowering plants and timing of flowering. The differences among years in these two characteristics serve to illustrate the mechanisms by which the florivores exert their effects on plant relative fitness and pollinator larval survivorship. In 2008, the relatively small number of plants flowering resulted in high densities of both leaf-footed bugs and *H. densus* beetles (Table 1). High densities of leaf-footed bugs would increase floral abscission and consequently reduce fruit set and the number of surviving pollinator larvae per plant. In 2008, the path from leaf-footed bugs to relative fitness was significant, negative, and strong when compared to other path coefficients. The increased floral abscission from leaf-footed bugs also likely outweighed any abscission caused by beetles and the beneficial effects of beetle feeding on moth eggs within flowers. The path coefficient for beetles to relative fitness was positive, but not significant, in 2008. In 2009, there was a threefold increase in the number of flowering plants. In this year, the average number of leaf-footed bugs per plant was reduced by threefold and they had no effect on relative fitness. In contrast, beetles had a significant positive effect on relative fitness. Average beetle densities per flower

were at levels that would not cause abscission (Table 2b), but could reduce the number of pollinator moth eggs (Segraves 2008). The same pattern was present in 2010, but the delayed flowering of plants further reduced the average number of beetles per flower to <1. Thus, even at low densities, beetles appear to increase seed set and relative fitness among individuals of *Y. filamentosa*.

In conclusion, the generalist florivore *L. phyllopus* and the specialist florivore *H. densus* can both influence plant female reproductive success and pollinator moth larval survivorship. The effects of these florivores are directly related to their densities on plants and within flowers. The year-to-year variation in the flowering phenology of *Y. filamentosa* contributes significantly to determining the densities of these florivores. In years with low numbers of flowering plants, the leaf-footed bug has a large negative effect on both plant seed set and pollinator larval survivorship. That effect decreases when there are more plants flowering in a year and leaf-footed bug densities per plant are reduced. Increases in the number of plants flowering have an opposite effect for *H. densus* beetles. Low densities of beetles results in significantly lower numbers of pollinator larval surviving within fruits of *Y. filamentosa*, which leads to increases in seed set. Taken together, the experimental manipulations and surveys of natural florivore abundances demonstrate the inconsistency of selection by florivores on plant flowering traits that contribute to female reproductive success. In terms of female reproductive success, the specialist florivore *H. densus* might be considered a mutualist rather than antagonist.

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