

Deborah L. Marr · Olle Pellmyr

Effect of pollinator-inflicted ovule damage on floral abscission in the yucca-yucca moth mutualism: the role of mechanical and chemical factors

Received: 19 August 2002 / Accepted: 17 April 2003 / Published online: 20 May 2003
© Springer-Verlag 2003

Abstract The long-term persistence of obligate mutualisms (over 40 Mya in both fig/fig wasps and yucca/yucca moths) raises the question of how one species limits exploitation by the other species, even though there is selection pressure on individuals to maximize fitness. In the case of yuccas, moths serve as the plant's only pollinator, but eggs laid by the moths before pollination hatch into larvae that consume seeds. Previous studies have shown that flowers with high egg loads are more likely to abscise. This suggests that yucca flowers can select against moths that lay many eggs per flower through selective abscission of flowers; however, it is not known how yucca moths trigger floral abscission. We tested how the moth *Tegeticula yuccasella* triggers floral abscission during oviposition in *Yucca filamentosa* by examining the effects of ovipositor insertion and egg laying on ovule viability and floral abscission. Eggs are not laid at the site of ovipositor insertion: we used this separation to test whether wounded ovules were more closely associated with the ovipositor site or an egg's location. Using a tetrazolium stain to detect injured ovules, we determined whether the number of ovipositions affected the number of wounded ovules in naturally pollinated flowers. Two wounding experiments were used to test the effect of mechanical damage on the probability of floral abscission. The types of wounds in these experiments mimicked two types of oviposition—superficial oviposition in the ovary wall and oviposition into the locular cavity—that have been observed in species of *Tegeticula*. The effect of moth eggs on ovule viability was

experimentally tested by culturing ovules in vitro, placing moth eggs on the ovules, and measuring changes in ovule viability with a tetrazolium stain. We found that ovules were physically wounded during natural oviposition. Ovules showed a visible wounding response in moth-pollinated flowers collected 7–12 h after oviposition. Exact location of wounded ovules relative to eggs and oviposition scars, as well as results from the artificial wounding experiments, showed that the moth ovipositor inflicts mechanical damage on the ovules. Significantly higher abscission rates were observed in artificially wounded flowers in which only 4–8% of the ovules were injured. Eggs did not affect ovule viability as measured by the tetrazolium stain. These results suggest that physical damage to ovules caused by ovipositing is sufficient to explain selective fruit abscission. Whether injury as a mechanism of selective abscission in yuccas is novel or a preadaptation will require further study.

Keywords Mutualism · Floral abscission · Ovule damage · Oviposition and selective floral abscission · Plant-pollinator interactions

Introduction

In order for mutualistic interactions to be evolutionarily stable, there should be either extrinsic (predators or abiotic factors) or intrinsic factors (mechanisms related directly to the interaction) that prevent excessive exploitation by the interacting species (Bull and Rice 1991; Connor 1995; Herre 1999). In the obligate mutualism between yuccas and yucca moths, yucca moths serve as the only documented pollinator, and the moth larvae must consume developing yucca seeds to complete development. The larvae consume a small fraction of the seeds; hence both species benefit from the interaction. Therefore, in yuccas the risk for excessive exploitation revolves around a fundamental conflict between the reproductive interests of the partners. Plants have higher fitness when fewer seeds are eaten, yet a female moth could increase

D. L. Marr (✉) · O. Pellmyr
Department of Biology, Vanderbilt University,
Nashville, TN 37235, USA
e-mail: dmarr@iusb.edu
Tel.: +1-574-2376564, Fax: +1-574-2376589

Present address:

D. L. Marr, Department of Biological Sciences,
Indiana University, South Bend, IN 46634, USA

Present address:

O. Pellmyr, Department of Biological Sciences,
University of Idaho, Moscow, ID 83844, USA

her fitness by laying more eggs per fruit until the point at which food resources become limited for her larvae.

Floral abscission is one intrinsic mechanism that reduces the chance of exploitation by some species of yucca moths. Many plants produce more flowers than can possibly be matured into fruit, and selective maturation of fruit is considered one of the major ways that a plant can control the quality of offspring (Stephenson 1981). The range of fruit:flower ratios varies widely among plant families with yuccas being towards the extreme end of species with very low fruit:flower ratios. Typically, fewer than 10% of the flowers produced by *Yucca* species mature into fruit (Udovic 1981; Addicott 1986, 1998; Huth and Pellmyr 1997), and this percentage of fruit set is low compared to other members of Agavaceae (mean \pm SE of 26.5 \pm 14.8) based on species collated by Sutherland and Delph (1984). Floral abscission is a nonrandom process in yuccas and, like many plant species, which fruit are retained depends upon pollen quantity (Huth and Pellmyr 1997), pollen genotype (Huth and Pellmyr 2000), and floral position (Aker 1982; Huth and Pellmyr 1997; Humphries and Addicott 2000). In addition to the importance of moth pollination behavior, oviposition behavior also affects fruit maturation. For instance, in *Yucca filamentosa* and *Hesperoyucca whipplei*, flowers with high egg loads are more likely to be abscised, which kills all of the eggs in the flower (Pellmyr and Huth 1994; Richter and Weis 1995). Selective maturation of fruit selects against moths that lay many eggs per flower and appears to be a critical factor in the stability of this mutualism.

At least two aspects of moth oviposition behavior have been suggested to trigger floral abscission. The first idea arose from the observation that fruit pollinated by yucca moths often have a characteristic constriction due to ovules that do not mature into seed. Riley (1892) noted that in *Y. filamentosa* two or more adjacent ovules were affected in the region of oviposition punctures. More recently, other authors have suggested that physical damage to the ovary during oviposition may be responsible for abscission (Fuller 1990; Humphries and Addicott 2000). In contrast, results from other studies have suggested that the presence of eggs is more strongly correlated with abscission (Pellmyr and Huth 1994; Wilson and Addicott 1998). There are few published data that distinguish between the effect of oviposition and the effect of eggs on ovule development and floral abscission. In the last decade, understanding the mechanism of how yucca moths trigger floral abscission has become even more intriguing in light of recent systematic and ecological studies of *Tegeticula* spp. that differ in oviposition behavior. Moth species that oviposit directly into the locule trigger floral abscission, whereas moth species that oviposit into the style or superficially into the fruit wall do not trigger floral abscission (Addicott and Bao 1999; Pellmyr and Leebens-Mack 2000). Understanding the mechanism behind why high egg loads and locule oviposition trigger floral abscission is important in addressing questions regarding how this mutualism

evolved, and provides more precise identification of the traits that affect whether the outcome of the interaction is antagonistic or mutualistic.

We tested several possible mechanisms that might explain how *T. yuccasella*, a locule-ovipositing species, triggers floral abscission in *Y. filamentosa*. Over 95% of floral abscission occurs before eggs hatch; therefore, larval feeding does not trigger floral abscission (Pellmyr and Huth 1994). As mentioned above, ovules do not develop in the region of oviposition (Riley 1892). Interference with ovule development could serve as a cue for abscission because fruits with fewer developing seeds are more likely to abscise (Huth and Pellmyr 2000). Taken together, these observations suggest that the trigger for floral abscission occurs within hours after moth oviposition. In addition, moths that oviposit superficially do not trigger the abscission response. Therefore, we focused on quantifying the specific type of damage that occurs to the ovary and ovules during oviposition, and on distinguishing between the effects of oviposition and presence of moth eggs on ovule development and floral abscission. Specifically, we tested the following questions. First, what type of mechanical damage occurs during natural oviposition? Second, is superficial mechanical damage to the ovary wall or mechanical damage within the locule sufficient to trigger floral abscission before eggs hatch? Third, are chemical secretions from the female moth necessary to disrupt ovule development or trigger abscission? Insect secretions, particularly the effects of plant piercing/sucking gall insects, have been shown to affect plant metabolism and alter hormonal balance enough to cause floral abscission (reviewed in Hori 1992; Sallabanks and Courtney 1992). Last, does the presence of moth eggs disrupt ovule development? The presence of moth eggs could potentially either alter the hormonal balance of the flower or directly kill ovules.

Materials and methods

Study species

The pollinator, *T. yuccasella* (Riley) sensu stricto (Lepidoptera: Prodoxidae), is a member of a large species complex (Pellmyr 1999), and was the only pollinator species observed at all sites in this study. Moths became active around dusk and remained active for 2–4 h. A female collects the sticky pollen with unique maxillary tentacles and compacts it into a loose pellet that is held under the head. After a female has collected pollen, she oviposits into a yucca ovary, then takes some of the pollen from her batch and actively pushes it into the stigmatic cavity. A female may oviposit into the ovary and pollinate a flower several times before walking or flying to another flower to repeat the procedure (Riley 1873; Pellmyr 1997). If an egg is laid, only one egg is laid per oviposition (Huth and Pellmyr 1999); females fail to lay an egg in over 30% of oviposition attempts (Pellmyr and Huth 1994). Adult moths typically live <5 days (Kingsolver 1984) and may drink water and trace amounts of nectar that sometimes collect at the ovary base, but otherwise do not feed as adults (Riley 1892; Pellmyr 1999).

Yucca filamentosa L. (Agavaceae) is a perennial native to the coastal areas of the southeastern United States and has been spread by humans throughout the eastern United States since the 1800s

(Riley 1892; Trelease 1902; Gleason and Cronquist 1991). Plants can reproduce both clonally, forming clusters of rosettes, and sexually through seed production. Each rosette can produce 100–475 flowers on a single, 1- to 2-m-tall paniculate inflorescence. Flowers open in the evening, anthers dehisce just before dusk, and stigmas are receptive upon flower opening. Flowers on an inflorescence open over the course of 10–20 days, and each flower is receptive to pollen for 1–2 days, but second-day flowers are typically rejected by pollinators (Huth and Pellmyr 1997). Capsular fruits mature and dehisce 6–8 weeks after pollination, producing up to 300 seeds per fruit.

Wounding response in moth-pollinated flowers

Ovule damage caused by oviposition was measured using a tetrazolium stain. 2,3,5-triphenyl tetrazolium chloride (TTC) serves as an electron acceptor for dehydrogenase enzyme systems and changes from a colorless solution to carmine red color when reduced (Roberts 1951). Normal viable embryo tissues develop a pink-red tint, recently injured living tissues develop a deep red stain, and critically injured or dead tissues remain white (Moore 1972). Thus, tetrazolium-staining patterns can indicate the presence, extent, and seriousness of damage from mechanical injuries (Moore 1972). Thirty flowers were collected from 15 plants 7–12 h after they had been pollinated by *T. yuccasella* on 23–26 June 1998 at Spring Grove Arboretum, Cincinnati, Ohio (39°06'N, 84°30'W). Flowers were kept at 4°C until they could be processed. Yucca flowers have three locules that each contain two rows of ovules, one row per locellus. Under a dissecting microscope using a scalpel and dissecting pins, the entire row of ovules was removed from a locellus and placed in a well with 0.1% TTC stain (Kearns and Inouye 1993). Ovules were allowed to stain for 30 min, or until the ovules had started to turn color, then were immediately scored for intensity of staining under a dissecting microscope. The position of eggs and scars relative to the staining response of the ovules was recorded. Scars can be clearly seen on the inside wall of the ovary because distinctive tear-shaped scar tissue forms around the scar. In addition, an impression of each ovule is also present on the inside wall of young ovaries. Thus, by studying the interior ovary wall alongside the stained ovules, we could accurately record which ovules were near the point at which the ovipositor entered the ovary, the position of the egg on the ovules, and the wounding response of the ovules. The difference between ovules with a wounding response and normal staining color was distinctive, as long as ovules were not overstained (Fig. 1). Flowers were processed as quickly as possible, usually within 24–48 h after collecting, but flowers could be stored up to 5 days at 4°C without a noticeable change in staining response.

This method provided information about the effect of *T. yuccasella* oviposition on the number of damaged ovules and distance between eggs and scars relative to damaged ovules. Distance was determined by counting the number of ovules between each egg and the nearest wounded ovule, and likewise the number of ovules between each scar and the nearest wounded ovule. This method of calculating distance provides a minimum estimate of distance for both eggs and scars because only the shortest possible distance was recorded for each egg. The average distance for eggs and scars within a flower was calculated by adding the distance for each egg (or each scar) and dividing by the number of eggs (or scars) laid in that flower. A paired *t*-test was used to compare average distance between wounded ovules and scars with that of distance to eggs. Data met assumptions of normality and heterogeneity of variances. A forward multiple regression was used to analyze the relationship between number of wounded ovules per flower and number of scars or eggs per flower. Number of scars and number of eggs are correlated. To determine whether it was necessary to retain both variables in the regression, the effect of the first variable (scar) on R-square was determined, then the second variable (egg) was entered into the equation to determine whether the addition of this variable significantly added

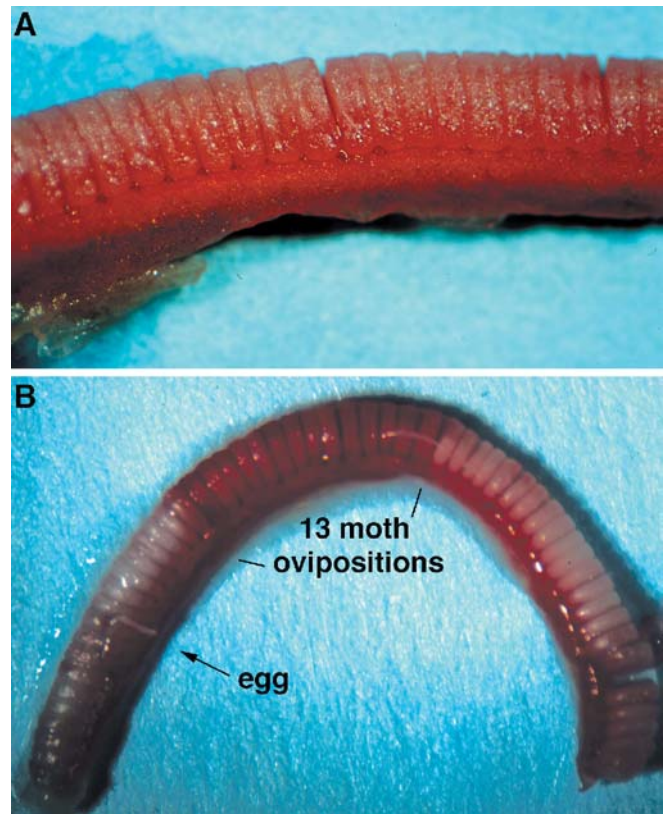


Fig. 1 **A** Ovules showing tetrazolium staining pattern from no moth ovipositions. Ovules stained with 0.1% tetrazolium. **B** Ovules showing staining pattern from 13 moth ovipositions. Several threadlike white eggs are also visible in the photograph

to the R-square value (Zar 1996; SPSS 1999). Number of eggs was non-significant and was dropped from the equation.

Artificial wounding experiments

To test the effect of mechanical damage caused by oviposition (in contrast to damage induced by either eggs or some factor introduced by the moth during oviposition), an artificial ovipositor was constructed that mimicked oviposition damage. The artificial ovipositor consisted of a minuten insect dissecting pin attached to a wooden matchstick and positioned at an angle similar to the angle in which moths oviposit into the ovary. The diameter of the dissecting pin was 0.11 mm and the diameter of *T. yuccasella*'s ovipositor including the maximum height of the keel from the study area is 0.12 ± 0.005 mm, $n=10$ (mean \pm SD) (Pellmyr 1999). The twisting motion a female uses to insert and remove her ovipositor was difficult to imitate given the rigidity of the pin and matchstick (and lack of nerve sensors to determine when one had entered the locule).

Given that some species of *Tegeticula* lay their eggs within the locule and other species lay their eggs superficially in the ovary wall, we did two wounding experiments: one experiment tested the effect of superficial mechanical wounding on floral abscission, and a second experiment tested the effect of 'deep' wounding on floral abscission. In the superficial wounding experiment, superficial wounds were applied by adjusting the artificial ovipositor to 1.2 mm so that the ovary wall was pierced. Ovules were not deeply pierced in this treatment. The superficial wounding experiment was done in a common garden at Vanderbilt University in May/June 1998. In the area of this study, *T. yuccasella* oviposits in the middle region of the ovary where ovary wall thickness of *Y. filamentosa* is

1.2±0.02 mm, $n=50$ flowers (mean±SE) (Huth and Pellmyr unpublished data). Treatments were applied to a total of 2,000 flowers on 13 plants. Each plant received four wounding treatments (0, 6, 12, or 24 wounds per flower); within plants, each treatment was applied to approximately 38 flowers. This number of wounds is within the natural range of oviposition scars observed in *Y. filamentosa* (range 1–32; Pellmyr and Huth 1994). The deep wounding experiment, referred to as ovule-wounding, consisted of adjusting the artificial ovipositor to 1.7 mm so that both the ovary wall and ovules were pierced. The ovule-wounding experiment was done in June 1999, which was a poor flowering year. Therefore, treatments were applied to five plants in the common garden at Vanderbilt, and to six plants in a secluded cedar glade located in central Tennessee (Rutherford County, 36°02'N, 86°24'W). Treatments were applied to a total of 1,320 flowers on 11 plants. Each plant received three wounding treatments (0, 12, or 24 wounds per flower); each treatment was applied to approximately 40 flowers within a plant.

In both wounding treatments, the effects of pollen genotype and flower position on floral abscission (Huth and Pellmyr 1997, 2000) were controlled by self-pollinating all flowers and randomizing treatments within branches. All four treatments were applied within a branch by randomly assigning one of four flowers on the branch to a treatment using a random number table. Branches that did not have four flowers available for pollination were paired with an adjacent branch, such that each day all four treatments were represented on either a single branch or two adjacent branches. At the whole plant level, care was taken to apply wounding treatments each day to an equal number of flowers. Flowers located at the extreme tip and basal portion of the branch were avoided due to the high probability of fruit set in the tip and low probability of fruit in the basal flowers (Huth and Pellmyr 1997). In addition, each inflorescence was covered with bridal veil netting (1 mm mesh size) to prevent yucca moths from visiting the flowers and to limit floral damage caused by the beetle *Carpophilus melanopterus* (Coleoptera: Nitidulidae).

Analysis of variance was used to analyze the effect of wounding treatments on the proportion of fruit that developed to maturity. The ANOVA model included wounding treatment (fixed factor) and plant (random factor). Proportion of retained fruit was arcsine square-root transformed and met the assumptions of normality and homogeneity of variances. A similar ANOVA model was used to analyze the effect of wounding treatments on proportion of filled seeds per fruit; the model included wounding treatment (fixed factor) and plant (random factor). Proportion of filled seeds was arcsine square-root transformed, and data met assumptions of normality and homogeneity of variances (Zar 1996). Tukey post-hoc tests were used to compare treatments when overall treatment effects were significant.

Effect of eggs on ovules in culture

To separate the effect of oviposition scars from the effect of eggs on ovule development, we cultured young seeds in vitro, applied moth eggs to the developing seeds, and then tested the seeds for viability. Eighteen 1-day-old flowers were hand-pollinated and collected after 12 h from 18 different plants at Spring Grove Arboretum, Cincinnati, Ohio. A straw was placed over the ovary to prevent oviposition by moths. Ovules were removed from the ovary as described in the tetrazolium experiment, and were placed on media made from a modified recipe of Murashige basal media agar (Murashige 1974; Binh et al. 1990). The modified recipe contained 8% sucrose, 4.3 g Murashige and Skoog salts, 0.4 mg thiamine, 10 mg inositol, 2.0 mg IAA, and 1% agar per liter of media, and the pH was adjusted to 6.0. Previous experiments varying sucrose and IAA concentration showed that ovule growth was greatest and persisted for 2–3 weeks on this media (D. Marr, unpublished data). Eggs were dissected from the ovaries of *T. yuccasella* females and were placed on top of the ovules. Each flower received three egg treatments (0 eggs, 2 eggs, and 6+ eggs), and each treatment was applied to two rows of ovules within each flower. Moth eggs were

sticky, making them difficult to separate, so in the 6+ egg treatment we recorded the number of eggs actually applied to the ovules (range 5–10). No difference in ovule staining was observed among ovules in the 6+ treatment; therefore, we considered it as one group (high egg treatment). The agar plates with ovules and egg treatments were placed in the dark and kept at 24°C. After 2 days, ovules were placed in 0.1% tetrazolium stain and were scored for viability.

The effect of eggs on ovules in culture was measured qualitatively. The color of ovules located underneath the moth eggs was classified as white (indication of no respiratory activity), light pink (low respiration activity), pink (active respiration), or dark pink (wounding response). The color of ovules in the 0-egg treatment was used as the null expectation for color. The color of ovules exposed to eggs in the 2-egg and 6-egg treatments was compared visually to the expected values derived from the 0-egg treatment. Data were analyzed using chi-square analysis. All statistical analyses were performed using SPSS version 10 for Macintosh (SPSS 1999).

Results

Distinguishing between the effect of eggs and oviposition on ovules in moth-pollinated flowers

We recorded a total of 438 scars and 277 eggs laid by *T. yuccasella* on 30 flowers. The effect of scars and eggs can be separated to some extent because eggs are not laid upon each oviposition attempt and the placement of eggs is not necessarily at the point of the oviposition scar. Eggs can be laid up to 8–10 ovules away from the point of ovipositor insertion (Fig. 1). The number of wounded ovules per flower was significantly positively correlated with the number of oviposition scars per flower (Fig. 2). The distance between oviposition scars and wounded ovules was significantly less than the distance between eggs and wounded ovules (Fig. 3; paired t test=4.89, 13 df , $P<0.0001$). Based on the forward regression analysis, the number of wounded ovules was primarily due to scars, and presence of eggs did not have any significant

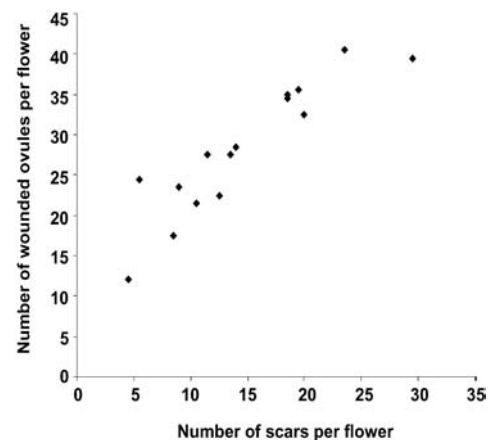


Fig. 2 Correlation between number of oviposition scars made by *Tegeticula yuccasella* and number of wounded ovules per flower. Adjusted R-square=0.82, $F_{1,13}=63.86$, $P<0.0001$; $n=15$ plants, two flowers per plant

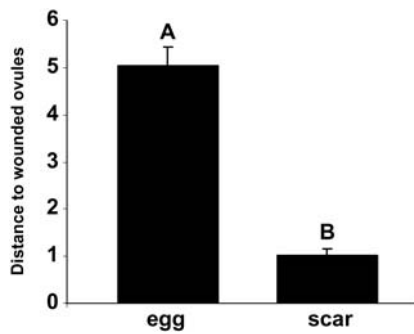


Fig. 3 Mean±SE distance between eggs and wounded ovules compared to mean distance between oviposition scars and wounded ovules. Distance is measured in number of ovules. Different letters indicate significant differences ($P<0.0001$)

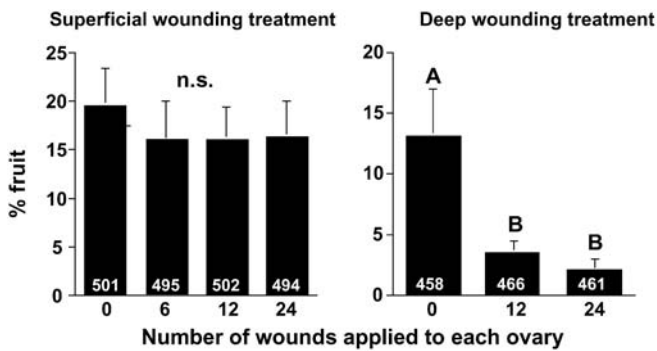


Fig. 4 Proportion of fruit retained per wounding treatment. Numbers of flowers pollinated per treatment are indicated on the bars. Analysis of variance results are presented in Table 2. Different letters indicate significant differences among treatments ($P<0.008$)

additional effect on the number of wounded ovules (Table 1).

Artificial wounding experiments and effect of eggs on ovules in culture

There were no significant differences among superficial wounding treatments (0, 6, 12, 24) in the percentage of fruit retained per treatment (Fig. 4; Table 2), or in the proportion of filled seeds per treatment (Fig. 5; Table 3). In contrast, deep wounding treatments had a significant effect on the proportion of fruit maturing (Fig. 4; Table 2), but there were no significant differences in the proportion of filled, viable seeds per fruit (Fig. 5; Table 3). For flowers that eventually matured into fruit, the day that the flower was pollinated did not differ among deep wounding treatments (Kruskal-Wallis $\chi^2=1.2$, $df=2$, $P=0.545$; mean±SE, 0 wound treatment 3.68 ± 0.11 days, 12 wound treatment 3.68 ± 0.10 days, 24 wound treatment 3.58 ± 0.10 days). Additionally, there was no difference in tetrazolium staining response among developing seeds that were exposed to 0 eggs, 2 eggs, or 6+ eggs (Table 4; $\chi^2=3.1$, $df=2$, $P=0.22$).

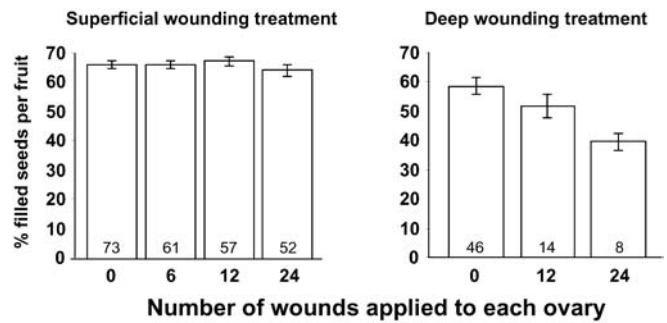


Fig. 5 Proportion of filled seeds per fruit in each wounding treatment. Numbers of fruits resulting from each treatment are indicated on the bars. Analysis of variance results are presented in Table 3

Table 1 Multiple forward regression analysis of the contribution of number of scars and number of eggs per flower on number of wounded ovules per flower. Adjusted $R^2=0.82$, ANOVA $F_{1,13}=63.86$, $P<0.0001$

Model	Standardized partial regression coefficient Beta	<i>t</i>	<i>P</i>
Constant		5.81	0.0001
Number of scars per flower	0.912	7.99	0.0001
Excluded variable			
Number of eggs per flower	-0.291	-0.791	0.444

Table 2 The results of analysis of variance for the effect of artificial superficial and deep wounding treatments on the proportion of matured fruit. The proportion of fruit was arcsine square-root transformed. The proportion of fruit per treatment is shown in Fig. 4

Source	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Superficial wounding				
Intercept	1, 12	6.875	60.01	0.0001
Wound treatment	3, 36	0.006	0.93	0.434
Plant	12, 36	0.115	17.26	0.0001
Deep wounding				
Intercept	1, 10	1.893	104.96	0.0001
Wound treatment	2, 20	0.115	10.11	0.001
Plant	10, 20	0.018	1.58	0.184

Table 3 The results of analysis of variance for the effect of artificial superficial and deep wounding treatments on the proportion of filled seed per fruit. The proportion of seed was arcsine square-root transformed. The proportion of filled seed per treatment is shown in Fig. 5

Source	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Superficial wounding				
Intercept	1, 10	202.45	1006.57	0.0001
Wound treatment	3, 271	0.052	0.561	0.434
Plant	10, 271	0.257	27.53	0.0001
Deep wounding				
Intercept	1, 12	17.57	263.96	0.0001
Wound treatment	2, 53	0.067	0.604	0.551
Plant	10, 53	0.129	11.62	0.0001

Table 4 Results of tetrazolium stain of ovules grown in culture and exposed to one of three egg treatments. The percentage of egg treatments that resulted in ovules in contact with the eggs turning white, light pink, or pink is shown. White indicated no respiration activity in ovule tissue, whereas light pink and pink indicated that ovule tissue was actively respiring. Dark pink coloration, an indication of injured tissue, was not observed in this experiment. *N* represents the number of plants

Number of <i>Tegeticula yuccasella</i> eggs applied to ovules	<i>n</i>	% white ovules	% light pink	% pink
0	18	17	44	39
2	18	6	61	33
6+	18	22	44	33

Discussion

Three lines of evidence support the hypothesis that oviposition by locule-ovipositing moths wounds ovules and that this physical damage is sufficient to trigger an abscission response in *Y. filamentosa*. First, in naturally pollinated flowers there is a strong correlation between the number of scars and the number of wounded ovules, as well as a short distance between scars and wounded ovules. This indicates that the point at which the ovipositor enters the locule is closely associated with the location of damaged ovules. Second, results of the artificial wounding experiments showed that physical wounding of ovules is sufficient to trigger an abscission response in *Y. filamentosa*. Punctures that only penetrated the ovary wall did not trigger abscission. Third, we did not observe any difference in viability between ovules exposed to eggs and those that were not in contact with eggs. Thus, we do not have any evidence at this point that eggs alone decrease ovule viability. However, studies of other insects have shown that additional secretions are added to the surface of eggs between the time the egg is released from the ovary and exits the ovipositor (Chapman 1998). Our method of collecting eggs directly from the ovaries does not address the possible effect that secretions added to eggs from accessory glands between the bursa and ovipositor affect ovule development.

Artificial wounding did not perfectly mimic moth oviposition. Based on the tetrazolium staining, each pin wound in the deep wounding treatment damaged 0.93 ± 0.05 ovules, whereas each oviposition scar damaged 2.38 ± 0.11 ovules (mean \pm SE). The damage radiates farther from the point of oviposition in moth-pollinated flowers compared to the point wounds caused by artificial wounding (Fig. 6). The broader spread of damage in natural ovipositions probably reflects the flexibility females have in where they place their egg, difference in timing of artificial and natural ovipositions, and difference in morphology of the artificial ovipositor. Successful oviposition attempts, in which an egg is laid, last about 4 times longer than unsuccessful attempts (Pellmyr, unpublished data). Each artificial oviposition lasted 10 s, approximately the time of an unsuccessful oviposition. The dissecting pin has a smooth surface

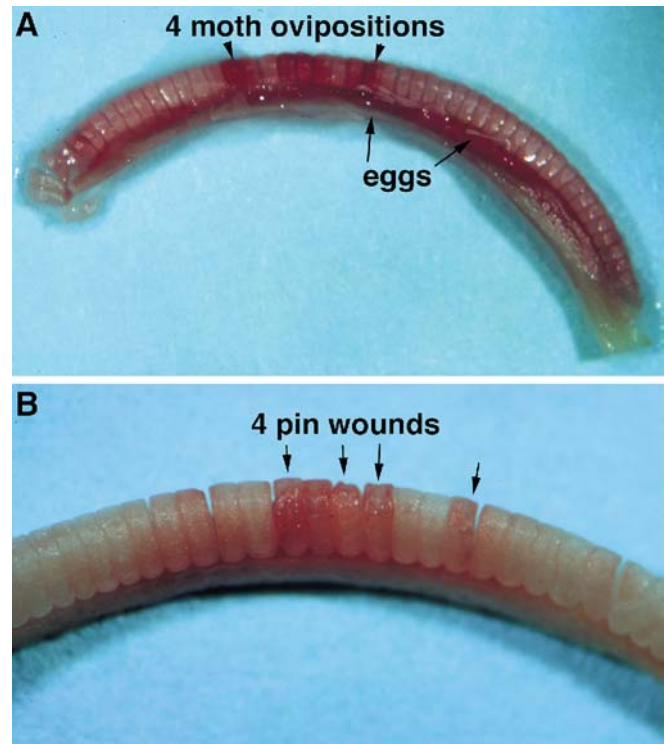


Fig. 6 **A** Ovules stained with 0.1% tetrazolium showing wounding response from four moth ovipositions. Eggs that were laid by the females are also visible in the photograph. **B** Ovules showing wounding response from four artificial 'deep' wounds

compared to the toothed edge of the moth ovipositor. Pollinators rarely pierce ovules deeply, but do move their ovipositor along ovules and can place eggs as far as 8 mm from the point where the ovipositor enters the locule. We have observed eggs located at the base of ovules, along the top, or between ovules (Figs. 1, 6). In short, artificial oviposition appeared to pierce ovules more deeply, but damaged fewer ovules per wound compared to natural oviposition. Damage caused by both ovipositors and artificial wounding killed ovules eventually, resulting in constricted fruits. Thus, fruits with many ovipositions have fewer ovules that mature into seeds than fruits with few ovipositions. Interestingly, we have observed that the degree of constriction in fruits varies among *Y. filamentosa* populations across the Midwest and southeastern United States, which may reflect variation in plant response to oviposition and variation in moth morphology, chemistry, and/or behavior.

The sensitivity of the abscission response in *Y. filamentosa* is striking. Significantly higher levels of abscission were detected in deep wounding treatments in which only 4% and 8% of the ovules were damaged. The physiological cause of abscission in fertilized flowers is typically attributed to resource limitation and/or changes in floral hormones (van Doorn and Stead 1997). Resource limitation can arise from temporal differences in ovule fertilization in which ovules that are fertilized first have an advantage in competing for limited carbohydrates

(Rodrigo and Herrero 1998) or mineral nutrition. In *Y. filamentosa*, differences in timing of fertilization does not explain the differences in wounding treatments because equal numbers of treatments were applied each day that first-day flowers were available. Furthermore, for flowers that eventually matured into fruit, the day that the flower was pollinated did not differ among deep wounding treatments. In other words, plants rejected flowers with high numbers of wounds whether the flowers opened early, middle, or late in the flowering phenology of that individual. The ability of the developing fruit to serve as a resource sink due to differences in number of wounded ovules is one possible physiological explanation for the abscission response. One hormone that may be particularly important in this interaction is ethylene. Ethylene has a major role in floral abscission in many species (Addicott 1982; van Doorn and Stead 1997), and is commonly involved in wounding responses (Salisbury and Ross 1996). Thus, the rapid abscission response to a high number of ovipositions may result from differences in resource acquisition and/or an increase in ethylene production due to wounding. Other hormones may be involved as well. In addition, wounds can alter action potentials, which are important in both inter- and intracellular communication (Davies 1987; Meyer and Weisenseel 1997). It is possible that oviposition wounds are a sufficient signal to directly trigger floral abscission, and that hormones play a minimal role in the response. It is unknown whether the sensitivity of *Y. filamentosa* to ovule wounding is a response common to many species.

Yuccas are not the only species in which insects can trigger floral abscission. Selective abscission of fruits damaged by insects has been reported for many plant species (Janzen 1971; reviewed in Sallabanks and Courtney 1992). A survey of 15 studies representing insects from the orders Coleoptera, Diptera, Hemiptera, and Lepidoptera showed that insects ovipositing or feeding on flowers increased the rate of floral abscission, although at least one study has shown that insect oviposition behavior can increase fruit retention (Brody and Morita 2000). Five of the 15 studies specifically tested how insects caused floral abscission. The mechanisms included indirect triggers of floral abscission by introducing fungi that caused fruit rot (Keck 1934), and direct mechanisms such as larval feeding damage (King and Lane 1969; Levine and Hall 1978; Hori et al. 1987; Cunningham 1997). Abscission triggered by larval feeding was caused by proteins produced by larvae in the fruit (King and Lane 1969; King 1973), production of pectinases and cellulases in saliva by larvae (Levine and Hall 1978), the formation of galls (Anderson 1989), or by altering plant hormones such as increasing levels of IAA-oxidase in response to larval feeding (Hori et al. 1987; Hori 1992). Damage during oviposition was not reported as a cause of floral abscission in these studies. In the obligate mutualism between figs and fig wasps, wasps directly oviposit into ovaries and destroy ovules, but this behavior apparently has no effect on the probability of fig abscission (Herre 1999).

The results of this study enable us to pinpoint more specifically the traits involved in stabilizing the interaction between locule-ovipositing *Tegeticula* species and yuccas. The majority of *Tegeticula* species oviposit directly into the locule (8 of 11 described pollinator species within the *yuccasella* complex). In *T. yuccasella*, a female only lays one egg per oviposition attempt (Huth and Pellmyr 1999) and no studies to date of locule-ovipositing species have shown that multiple eggs can be laid per oviposition. However, there are three species that superficially lay eggs into either the ovary wall or at the base of the style (Pellmyr 1999; Segraves, unpublished data). Interestingly, superficial oviposition does not affect floral abscission (Addicott and Bao 1999; this study). Variability in the mode of moth oviposition suggests that it is not a fixed trait that limits the range of possible outcomes in yucca-yucca moth interactions. Further work on other *Yucca* species and on species throughout the Agavaceae will help address whether floral abscission triggered by ovule injury represents a preadaptation or a direct adaptation to the mutualism.

Acknowledgements We thank Lindsey Elms for help with the artificial wounding experiments, Chad Huth for his advice in the early stages of this project, Holly Bonar and Michael Lacey for help with seed counting, and Kari Segraves and two anonymous reviewers for comments on an earlier version of this manuscript. This work was supported by NSF (DEB 95-09056) and a Natural Science Grant from Vanderbilt University to O. Pellmyr.

References

- Addicott FT (1982) Abscission. University of California Press, Berkeley
- Addicott JF (1986) Variation in the costs and benefits of mutualism: the interaction between yuccas and yucca moths. *Oecologia* 70:486–494
- Addicott JF (1998) Regulation of mutualism between yuccas and yucca moths: population level processes. *Oikos* 81:119–129
- Addicott JF, Bao T (1999) Limiting the costs of mutualism: multiple modes of interaction between yuccas and yucca moths. *Proc R Soc Lond Ser B* 266:197–202
- Aker CL (1982) Regulation of flower, fruit and seed production by a monocarpic perennial, *Yucca whipplei*. *J Ecol* 70:357–372
- Anderson AN (1989) Impact of insect predation on ovule survivorship in *Eucalyptus baxteri*. *J Ecol* 77:62–69
- Binh LT, Muoi LT, Oanh HTK, Thang TD, Phong DT (1990) Rapid propagation of *Agave* by in vitro tissue culture. *Plant Cell Tissue Organ Cult* 23:67–70
- Brody AK, Morita SI (2000) A positive association between oviposition and fruit set: female choice or manipulation? *Oecologia* 124:418–425
- Bull JJ, Rice WR (1991) Distinguishing mechanisms for the evolution of cooperation. *J Theor Biol* 149:63–74
- Chapman RF (1998) The insects: structure and function, 4th edn. Cambridge University Press, Cambridge
- Connor RC (1995) The benefits of mutualism: a conceptual framework. *Biol Rev* 70:427–457
- Cunningham SA (1997) Predator control of seed production by a rain forest understory palm. *Oikos* 79:282–290
- Davies E (1987) Action potentials as multifunctional signals in plants: a unifying hypothesis to explain apparently disparate wound responses. *Plant Cell Environ* 10:623–631
- Doorn WG van, Stead AD (1997) Abscission of flowers and floral parts. *J Exp Bot* 48:821–837

- Fuller OS (1990) Factors affecting the balance of cooperation and conflict between the yucca moth, *Tegeticula yuccasella*, and its mutualist, *Yucca glauca*. Ph.D. dissertation, University of New Mexico, Albuquerque
- Gleason HA, Cronquist A (1991) Manual of vascular plants of northeastern United States and adjacent Canada, 2nd edn. New York Botanical Garden, Bronx
- Herre EA (1999) Laws governing species interactions? Encouragement and caution from figs and their associates. In: Keller L (ed) Levels of selection in evolution. Princeton University Press, Princeton, NJ, pp 209–237
- Hori K (1992) Insect secretions and their effect on plant growth, with special reference to Hemipterans. In: Shorthouse JD, Rohfritsch O (eds) Biology of insect-induced galls. Oxford University Press, Oxford, pp 157–170
- Hori K, Torikura H, Kumagai M (1987) Histological and biochemical changes in the tissue of pumpkin fruit injured by *Lygus disponi* Linnavuori (Hemiptera: Miridae). Appl Entomol Zool 232:259–265
- Humphries SA, Addicott JF (2000) Regulation of the mutualism between yuccas and yucca moths: intrinsic and extrinsic factors affecting flower retention. Oikos 89:329–339
- Huth CJ, Pellmyr O (1997) Non-random fruit retention in *Yucca filamentosa*: consequences for an obligate mutualism. Oikos 78:576–584
- Huth CJ, Pellmyr O (1999) Yucca moth oviposition and pollination behavior is affected by past flower visitors: evidence for a host-marking pheromone. Oecologia 119:593–599
- Huth CJ, Pellmyr O (2000) Pollen-mediated selective abortion in yuccas and its consequences for the plant-pollinator mutualism. Ecology 81:1100–1107
- Janzen DH (1971) Seed predation by animals. Annu Rev Ecol Syst 2:465–492
- Kearns CA, Inouye DW (1993) Techniques for pollination biologists. University Press of Colorado, Niwot
- Keck CB (1934) Relation of oviposition punctures of the Mediterranean fruit fly to the premature dropping of citrus fruits. J Econ Entomol 28:908–914
- King EE (1973) Endo-polymethylgalacturonase of boll weevil larvae, *Anthonomus grandis*: an initiator of cotton flower bud abscission. J Insect Physiol 19:2433–2437
- King EE, Lane HC (1969) Abscission of cotton flower buds and petioles caused by protein from boll weevil larvae. Plant Physiol 44:903–906
- Kingsolver RW (1984) Population biology of a mutualistic association: *Yucca glauca* and *Tegeticula yuccasella*. Ph.D. dissertation, University of Kansas, Lawrence
- Levine E, Hall FR (1978) Pectinases and cellulases from plum curculio larvae: possible causes of apple and plum fruit abscission. Entomol Exp App 23:259–268
- Meyer AJ, Weisenseel MH (1997) Wound-induced changes of membrane voltage, endogenous currents, and ion fluxes in primary roots of maize. Plant Physiol 114:989–998
- Moore RP (1972) Effects of mechanical injuries on viability. In: Roberts EH (ed) Viability of seeds. Syracuse University Press, Chapman and Hall, UK pp 94–113
- Murashige T (1974) Plant propagation through tissue cultures. Annu Rev Plant Physiol 25:135–66
- Pellmyr O (1997) Pollinating seed eaters: why is active pollination so rare? Ecology 78:1655–1660
- Pellmyr O (1999) Systematic revision of the yucca moths in the *Tegeticula yuccasella* complex (Lepidoptera: Prodoxidae) north of Mexico. Syst Entomol 24:1–28
- Pellmyr O, Huth CJ (1994) Evolutionary stability of mutualism between yuccas and yucca moths. Nature 372:257–260
- Pellmyr O, Leebens-Mack J (2000) Reversal of mutualism as a mechanism for adaptive radiation in yucca moths. Am Nat 156:S62–S76
- Richter KS, Weis AE (1995) Differential abortion in the yucca. Nature 376:557–558
- Riley CV (1873) On the oviposition of the yucca moth. Am Nat 7: 619–623
- Riley CV (1892) Some interrelations of plants and insects. Proc Biol Soc Wash 7:81–104
- Roberts LW (1951) Survey of factors responsible for reduction of 2,3,5-triphenyltetrazolium chloride in plant meristems. Science 113:692–693
- Rodrigo J, Herrero M (1998) Influence of intraovular reserves on ovule fate in apricot (*Prunus armeniaca* L.). Sex Plant Reprod 11:86–93
- Salisbury FB, Ross CW (1996) Plant physiology, 4th edn. Wadsworth, Belmont
- Sallabanks R, Courtney SP (1992) Frugivory, seed predation, and insect-vertebrate interactions. Ann Rev Entomol 37:377–400
- SPSS (1999) SPSS 10.0 syntax reference guide. SPSS Inc., Chicago
- Stephenson AG (1981) Flower and fruit abortion: proximate causes and ultimate functions. Annu Rev Ecol Syst 12:253–279
- Sutherland S, Delph LF (1984) On the importance of male fitness in plants: patterns of fruit-set. Ecology 65:1093–1104
- Trelease W (1902) The Yuccaeae. Mo Bot Garden 13th Annu Rep pp 27–133
- Udovic D (1981) Determinants of fruit set in *Yucca whipplei*: reproductive expenditure vs. pollinator availability. Oecologia 48:389–399
- Wilson RD, Addicott JF (1998) Regulation of mutualism between yuccas and yucca moths: is oviposition behavior responsive to selective abscission of flowers? Oikos 81:109–118
- Zar JH (1996) Biostatistical analysis, 3rd edn. Prentice Hall, Upper Saddle River