UNDERSTANDING STABILITY IN MUTUALISMS: CAN EXTRINSIC FACTORS BALANCE THE YUCCA–YUCCA MOTH INTERACTION?

Kari A. Segraves[1]

Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee 37235 USA

Abstract. Mutualisms form an integral part of many communities and play an important role in the maintenance and promotion of biodiversity. The inherent conflict of interest between mutualists suggests that partners should reduce the cost of the interaction whenever possible. As a result, defense mechanisms may evolve to prevent overexploitation by both partners. Selection for the evolution of defenses intrinsic to the interaction may be unnecessary, however, under particular ecological conditions. I examined the interaction between the pollinating yucca moth *Tegeticula cassandra* and its host plant *Yucca filamentosa* to determine whether extrinsic factors are important in promoting stability in this mutualism. Yuccas can selectively abscise fruit with high egg loads for moth species that damage ovules during oviposition into the locule. Female *T. cassandra*, however, lay eggs less than a millimeter beneath the tissue surface and fail to damage ovules, circumventing this mechanism of selective abscission. Consequently, yuccas are unable to regulate egg loads by *T. cassandra*, and moths are free to lay many eggs. Despite this, only 5–11% of *T. cassandra* eggs survive to exit the fruit as mature larvae. I tested the hypothesis that *T. cassandra* survivorship is regulated by the extrinsic factors dehydration and predation. While exclusion of potential predators seemed to have no effect on survivorship, moth eggs in flowers with experimentally increased vapor pressure had significantly greater survivorship compared to controls. Survivorship of a closely related locule-ovipositing species was unaffected by either predator exclusion or changes in vapor pressure. The results suggest that the stability of mutualisms can depend at least in part on extrinsic factors.

Key words: exploitation; obligate mutualism; plant–insect interactions; pollination; Prodoxidae; regulatory mechanisms; sanctions; selective abscission; stability; survivorship; Tegeticula; *yucca–yucca moth.*

INTRODUCTION

Mutualisms are interspecific interactions where both species benefit from participating in the interaction. These interactions carry both costs and benefits, and for this reason, a given association may fall along a continuum encompassing commensalism, mutualism, or antagonism. Theoretical work on the evolution of mutualisms suggests that these interactions are inherently unstable, subject to invasion by exploitative individuals who provide no benefit, yet take resources supplied by mutualists (Trivers 1971, Axelrod and Hamilton 1981, Bull and Rice 1991, Schwartz and Hoeksema 1998). Further studies have shown that the underlying conflict of interest between mutualists can shape or destabilize an interaction (Bronstein 1994, Pellmyr and Huth 1994, Thompson 1994, Herre and West 1997, Herre et al. 1999, Hibbett et al. 2000) and the outcome of a given interaction will depend in part on local ecological conditions (Cushman and Whitham 1989, Breton and Addicott 1992, Thompson and Pell-

myr 1992, Bronstein 1994, Del-Claro and Oliveira 2000, Morales 2000, Offenberg 2001). As a result, there may be specific mechanisms that help to promote stability by maximizing the benefits while minimizing the costs associated with the mutualism (Axelrod and Hamilton 1981, Murray 1985, Bull and Rice 1991, Johnson et al. 1997, Axén and Pierce 1998, Doebeli and Knowlton 1998, Schwartz and Hoeksema 1998, Addicott and Bao 1999, Bultman et al. 2000, Denison 2000, Sherratt and Roberts 2002).

Recent models of mutualism have revealed that the presence of sanctions or regulatory mechanisms preventing overexploitation can lead to stable equilibrium (Holland and DeAngelis 2001, 2002, Holland et al. 2002, West et al. 2002). For example, fig trees may avoid overexploitation by their pollinating fig wasps by producing flowers that fig wasps are unable to oviposit into or by limiting access into the specialized inflorescence (West and Herre 1994, Nefdt and Compton 1996, Herre 1999, Weiblen et al. 2001). In the yucca–yucca moth interaction, selective fruit abscission has been shown to prevent moths from laying so many eggs that all seeds are destroyed (Pellmyr and Huth 1994, Addicott 1998, Wilson and Addicott 1998). These examples highlight factors intrinsic to the interaction (between mutualist partners) that regulate the mutualism. While the evolution of intrinsic regulatory

[1] Present Address: University of Idaho, Department of Biological Sciences, Moscow, Idaho 83844-3051 USA. E-mail: ksegrave@uidaho.edu
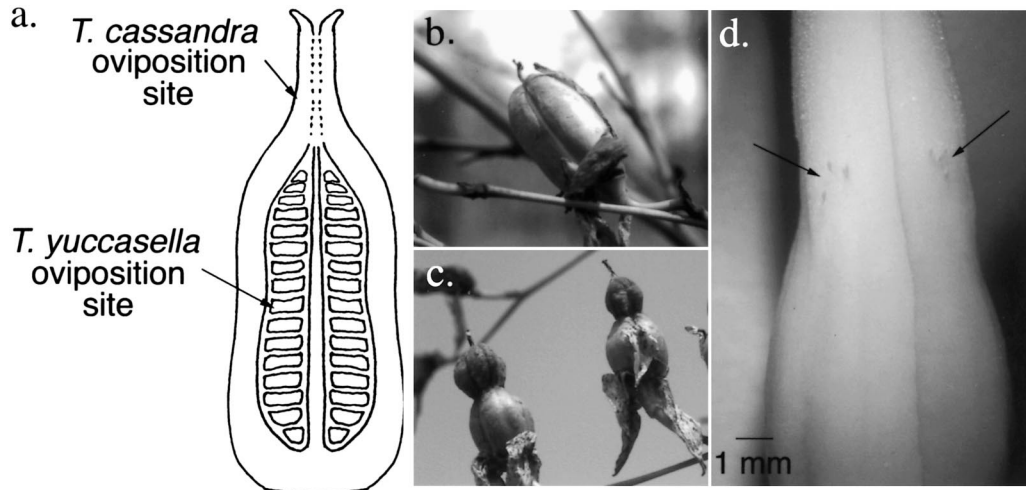
FIG. 1. *Tegeticula cassandra* and *T. yuccasella* oviposition sites and resulting fruit shapes. (a) Diagram of longitudinal section through a *Y. filamentosa* pistil. *Tegeticula cassandra* oviposits superficially in the base of the style, whereas *T. yuccasella* deposits eggs into the locule, often wounding ovules in the process. (b) Fruit resulting from *T. cassandra* pollination. Note the characteristic "straight" shaped fruit. (c) Fruit resulting from *T. yuccasella* pollination showing characteristic constriction at the point of oviposition. (d) Photograph of *T. cassandra* oviposition scars. Eggs are often clustered and are laid extremely close to the surface. Photograph courtesy of O. Pellmyr.

mechanisms may be particularly important in such highly specialized mutualisms, their utility in providing generalizations for less specialized mutualisms is likely to be limited. Even for highly specialized interactions there may be factors extrinsic to the interaction that are effective at preventing overexploitation. For example, abiotic factors or a species external to the pairwise interaction may be equally effective. One of the keys, then, to understanding the evolution of mutualistic interactions is to determine whether the mechanisms involved in regulating mutualisms are intrinsic or extrinsic to the interaction and the specific conditions that favor stability.

The yucca–yucca moth association is among the classic examples of mutualism. This interaction is an obligate pollination mutualism that has persisted for over 40 million years (Pellmyr and Leebens-Mack 1999). Yucca moths are the sole pollinators of yuccas, and yucca seeds serve as food for developing moth larvae. Female moths lay their eggs into yucca flowers and actively pollinate, and the larvae subsequently consume a fraction of the developing yucca ovules. Phylogenetic analyses have revealed a recent radiation of yucca moths harboring a diversity of oviposition habits based on where female pollinators lay their eggs (Pellmyr 1999).

Two species of pollinating yucca moths in the southeastern United States utilize the host plant *Yucca filamentosa* and they differ in oviposition habit (Pellmyr 1999 and Fig. 1a–c). *Tegeticula yuccasella* is a widespread species, occurring throughout the eastern United States. The female *T. yuccasella* oviposits into the locule by piercing through the ovary wall to deposit her eggs next to the developing ovules. As the female punc-tures the ovary wall, ovules are wounded, causing rapid ovule death that results in a characteristic constriction of the fruit (Marr and Pellmyr 2003). Because yuccas selectively abscise flowers with high numbers of damaged ovules (Marr and Pellmyr 2003), flowers where *T. yuccasella* have laid many eggs are selectively dropped from the plant, killing all eggs and larvae within them (Pellmyr and Huth 1994). Hence, selective abscission is one mechanism by which yuccas can regulate the number of *T. yuccasella* eggs placed within their flowers and prevent overexploitation by the moths. The second moth species, *T. cassandra*, has a more restricted range, occurring only in Florida and Georgia. This species lays its eggs superficially in the base of the style, just piercing the tissue surface without damaging ovules. Eggs are deposited at depths of a few tenths of a millimeter, and frequently project from the tissue surface. As a result, the known regulatory mechanism for the locule-ovipositing species does not work for *T. cassandra*. Although *T. cassandra* lay many more eggs per flower than *T. yuccasella* (mean $\pm$ 1 SE = 6.68 $\pm$ 0.20 eggs per flower for *T. cassandra* vs. 2.92 $\pm$ 0.13 for *T. yuccasella*, $P < 0.0001$, $\chi^2 = 230.64$, df = 1), few *T. cassandra* eggs survive to exit the fruit as mature larvae. This paper explores the possibility of alternative factors that may help to prevent overexploitation by this superficially ovipositing moth species.

Because *T. cassandra* eggs are relatively exposed in the style tissue, I hypothesized that extrinsic factors may be important in regulating the mutualism between *T. cassandra* and *Y. filamentosa*, whereas extrinsic factors should be less important for the mutualism between *T. yuccasella* and *Y. filamentosa*. The superficial place-

ment of *T. cassandra* eggs may expose them to attack by predators and to dehydration. Specifically, like the eggs of all basal Lepidoptera, those of *Tegeticula* are relatively unprotected in that they lack a protective lamellar layer C-3 that reduces dehydration in more derived Lepidoptera (Fehrenbach, *in press*). The C-3 layer is the outer layer of the chorion that has architecture similar to the arthropod cuticle (Fehrenbach, *in press*). This layer has been shown to be important in physical protection from dehydration (Chauvin and Barbier 1972), and potentially predation (Fehrenbach, *in press*). Using the locule-ovipositing *T. yuccasella* for comparison, I addressed several questions on the factors contributing to stability in the interaction between *T. cassandra* and its yucca host. In contrast to the *T. yuccasella*– *Y. filamentosa* mutualism, is the mutualism between *T. cassandra* and *Y. filamentosa* possibly regulated by extrinsic factors? If so, what are the factors involved? Is egg predation important in reducing survivorship of *T. cassandra*? Does flower microclimate influence the survivorship of *T. cassandra*?

METHODS

Parallel studies were conducted at Archbold Biological Station (henceforth Archbold; 27°11′18″ N, 81°20′16″ W) near Lake Placid, Florida, and at Eglin Air Force Base (henceforth Eglin; 30°44′00″ N, 86°22′00″ W) near Crestview, Florida, to compare differences in larval survivorship between the superficial-ovipositing moth *T. cassandra* and the locule-ovipositing moth *T. yuccasella*. *Yucca filamentosa* at Archbold are primarily pollinated by *T. cassandra*, with a few visits early in the season by *T. yuccasella*. The exclusive pollinator at Eglin is *T. yuccasella*. This study was conducted over the course of the flowering period of the host during April, May, and June of 2000–2002.

Measurements of survivorship

Larval survivorship on individual fruit was measured by comparing the number of oviposition scars on a flower to the number of mature larvae that survived to exit the fruit. Oviposition scars were counted with the assistance of a 20× hand lens (Fig. 1d). The day following anthesis, flowers were individually labeled with the scar count. To avoid the possibility of temporal variations in survivorship, flowers were labeled within 3 d of one another. Fruit were collected after 30–35 d and dissected to determine the number of mature larvae. Because yucca moths are not always successful in laying eggs during oviposition, an egg-to-scar ratio was determined for both moth species. I did this by collecting flowers that had been pollinated the previous night, counting the number of scars, and then dissecting each flower to determine the number of eggs laid. Scar counts and dissections were made using an Olympus SZ-PT dissecting microscope at 25× magnification (Olympus America, Melville, New York, USA). The

resulting egg-to-scar ratios were then used to generate a correction factor for the expected number of larvae. The slope of the best-fit line forced through zero was used as the correction factor. Survivorship was determined on a per-fruit basis by comparing the expected number of larvae to the observed number of larvae within fruit.

Flower microclimate

A flower microclimate experiment was conducted at Archbold and in the surrounding area of Lake Placid, Florida, in 2000 and 2002 to determine survivorship of *T. cassandra* in an experimentally altered microclimate. A parallel study on *T. yuccasella* was conducted at Eglin in 2001 and at a nearby site at Crestview, Florida, in 2002. Measurements of temperature and relative humidity were made on plants at Archbold and Eglin in 2002.

In order to determine whether abiotic factors were important in reducing survivorship of *T. cassandra* eggs and larvae, I altered flower microclimate by placing small mesh bags over individual flowers. These bags were predicted to provide a more favorable microclimate for superficially laid moth eggs by increasing relative humidity and decreasing temperature inside of flowers. Oviposition scars were counted on the day following anthesis, and then one of three different flower treatments was randomly assigned to each flower within a plant: no mesh bag (control), coarse-mesh bag (1-mm mesh size), or fine-mesh bag (0.2 mm mesh size). Coarse-mesh bag treatments were predicted to provide an environment more favorable than control flowers, but less favorable than the environment offered by fine-mesh bag treatments. Mesh bags held a single flower and were attached to flowers using garden twist-ties wrapped around the base of the flower pedicel. All three treatments were applied to each plant used in the microclimate experiment. That is, some flowers within a plant were left as controls, a subset were placed in coarse-mesh bags, and a third set were placed in fine-mesh bags. Treatments were applied to 18–30 plants per site per year, and temporal differences were avoided by applying treatments to flowers that opened within 3 d of one another during peak moth emergence.

These bags remained on the fruit until the larvae were mature after ∼30–35 d, at which time the fruits were collected and larval survivorship was determined following the procedures outlined above. Survivorship proportions were arcsine transformed and a one-way ANOVA was used to assess significant differences in survivorship across the three treatments. The unit of replication was the fruit. In cases where the data remained heteroscedastic, a Kruskal-Wallis test was used. All statistics were calculated using JMP 3.2.1 (SAS Institute 1998). Although 150 flowers were initially assigned to each treatment per site per year, the final sample sizes varied because yuccas retain <20% of their fruit (Aker 1982, Keeley et al. 1984, James et al.

1993, Pellmyr and Huth 1994, Richter and Weis 1995). Because fruit retention was unpredictable and often resulted in unbalanced treatments within plants (e.g., missing treatments), the analysis could not be blocked by plant. Comparisons were made on 125 (2000) and 134 (2002) fruit at Archbold and 95 (2001) and 69 (2002) fruit at Eglin.

Predator exclusion

The mesh bag treatments also prevented predator access to moth eggs. Ants are probably the main predators of mature yucca moth larvae (Riley 1873; E. Tepe and O. Pellmyr, *unpublished data*), and may also attack eggs. In addition to ants, parasitoids are known to be important natural enemies of bogus yucca moths in the closely related genus *Prodoxus*, and in some localities, parasitoids attack *Tegeticula* at low frequency (Force and Thompson 1984, Powell 1984; B. A. Crabb and O. Pellmyr, *unpublished manuscript*). An extensive four-year survey of *T. cassandra* and *T. yuccasella* in the southeastern United States, however, found no yucca moth parasitoids (D. M. Althoff, *unpublished data*). This demonstrates that parasitoid attack is extremely rare for these moths.

To ascertain the effect of ant predators on egg survivorship, ants were excluded from one-half of the plants in the microclimate experiment by placing a 3 cm wide ring of Tangle-Trap (The Tanglefoot Company, Grand Rapids, Michigan, USA) near the base of the inflorescence stalk on the day following anthesis. Once the trap was in place, all ants were removed from the portion of the inflorescence above the trap. Tangle-Trap creates an effective barrier for crawling insects. Dead ants were occasionally found in the trap ring and approaching ants avoided the trap. The Tangle-Trap ring was placed as far as possible from the flowers (1–2 m), and should not have affected measurements of microclimate. Larval survivorship for the control flowers (no bag) was used to assess whether ant predation influenced survivorship. For these analyses, I excluded measurements of survivorship for the other treatments (mesh bags) because the mesh bags also excluded predators and would not make an appropriate comparison. Differences in survivorship between predator treatments were assessed using a one-way ANOVA on the arcsine-transformed survivorship proportions. In cases where the data violated the assumptions of ANOVA, a Kruskal-Wallis test was used. The unit of replication was individual fruit. The predator exclusion experiment was conducted at Archbold in 2000 and 2002, and at Eglin in 2002. Comparisons of survivorship were made on 43 (2000) and 14 (2002) fruit at Archbold and 25 fruit at Eglin.

Measurements of microclimate

Although many abiotic factors were probably altered by the mesh bag treatments (e.g., light intensity, wind speed), I chose to focus on temperature and relative humidity as the most probable factors determining survivorship for *T. cassandra* because egg dehydration is likely to be the most important cause of mortality for superficially laid eggs. High heat and egg dehydration are critical sources of mortality in many insects (e.g., Krasnov et al. 2001, Guarneri et al. 2002, Juliano et al. 2002), including Lepidoptera (e.g., Chauvin and Barbier 1972, Ryan 1985, Fehrenbach, *in press*). Temperature and relative humidity were measured using HOBO weather stations fitted with small temperature/relative humidity probes (Onset Computer Corporation, Bourne, Massachusetts, USA). Weather stations measured $18 \times 23 \times 10$ cm with a maximum capacity of 15 channels and a memory capacity of 512K or 500 000 measurements. The stainless steel probes were 1.6×10.1 cm with an accuracy of $\pm3\%$ relative humidity and $\pm0.7°C$. Probes were placed directly inside of mesh bags with flowers or immediately outside of flowers lacking bags. Measurements were logged every 5 min for the first 7 d after flowers opened. This 7-d period encompasses the time frame that *T. cassandra* eggs require to hatch. Mean hourly temperature and relative humidity were calculated from the recordings and used to determine the hourly mean vapor pressure. Significant differences in vapor pressure were assessed using a multivariate repeated-measures design where time was the repeated measure. Uniform covariance was checked and in cases where the data violated this assumption, the Huynh-Feldt Epsilon adjusted degrees of freedom were used (Milliken and Johnson 1992).

Impact of larval feeding

To strengthen the connection between survivorship and plant reproduction, I determined seed consumption by *T. cassandra* larvae that survived to exit the fruit. Fruits were collected at Archbold late in the season after larvae had emerged. These fruits were collected from plants that remained unaltered and were not included in any of the above experiments. The number of larvae per fruit was determined by counting the distinctive exit holes and feeding paths left by emerging larvae. Fruit were dissected to determine the number of seeds consumed per larva. The total number of seeds per fruit was also recorded. *Tegeticula cassandra* feeding paths are readily identified by a characteristic feeding hole at the fruit apex where larvae chew into the locule. Larvae tunnel paths through the seed rows, and only consume a portion of an individual seed. Just prior to emergence, larvae spin cocoons within their feeding paths, so that damaged seeds cling together and are easily sorted from intact seeds. The proportion of seeds consumed per larva was determined by comparing the number of seeds consumed to the total number of seeds within the fruit (intact plus damaged). In total, I examined 34 fruit collected from five plants.

RESULTS

Oviposition success (eggs deposited per oviposition scar) for *T. cassandra* females was high, closely ap-
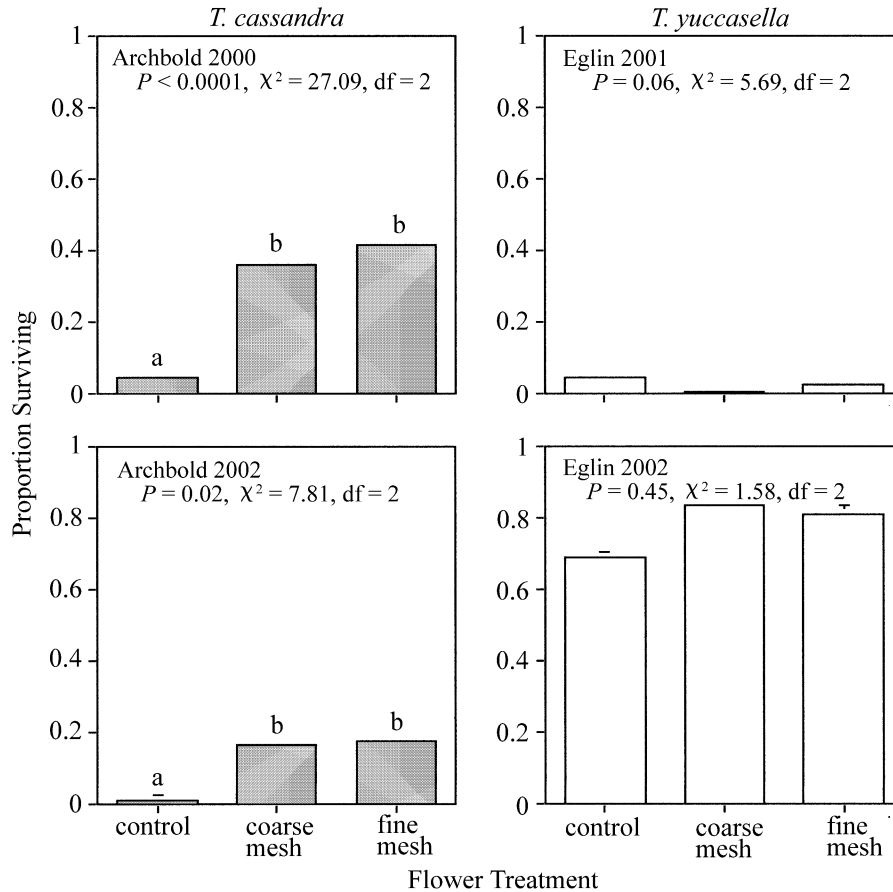
FIG. 2. Survivorship results for *T. cassandra* and *T. yuccasella*: back-transformed means and standard errors for *T. cassandra* (left) and *T. yuccasella* (right). Different letters over the bars indicate significant differences between treatments. For most bars, the standard errors are too small to be seen.

proximating a 1:1 ratio ($y = 0.96x$; $R^2 = 0.96$). Female *T. yuccasella* were far less successful, with eggs laid ~45% of the time ($y = 0.45x$; $R^2 = 0.22$). Consequently, the survivorship estimates for both moth species were corrected. For *T. cassandra*, 9 in 148 flowers had more eggs than oviposition scars, and 5 in 197 flowers pollinated by *T. yuccasella* had more eggs than scars. These results indicate that females occasionally deposit more than one egg during a single oviposition, or alternatively, that females sometimes pierce repeatedly in the same position on a flower. These events were rare, so another correction was not incorporated into the survivorship calculations. Survivorship of *T. cassandra* was normally distributed and was consistently higher in mesh bag treatments. There was no difference in survivorship between the two different mesh bag treatments (Fig. 2). *Tegeticula yuccasella* survivorship was not affected by treatment in either year (Fig. 2). Survivorship differed between years for both moth species ($P < 0.0001$, $F > 10.85$, for all), but there was no year by treatment interaction, demonstrating that the differences observed were consistent among years, although the magnitude of those differ-

ences varied. In addition, there was no difference in the distribution of the number of oviposition scars across treatments at either site ($P > 0.33$, $\chi^2 < 2.21$, df = 2 for all). For both moth species, survivorship was unaffected by the exclusion of potential ant predators ($P > 0.10$, $\chi^2 < 2.70$ df = 1 for all). Seed consumption by individual *T. cassandra* larvae was 15.23 $\pm$ 0.28% (mean $\pm$ 1 SE) of the seeds within a fruit (range 10.7–24.7% per larva, $N = 103$ larvae in 34 fruit).

Overall mean vapor pressure was significantly lower at the Eglin site (mean of 20.8 $\pm$ 0.18% vs. 30.32 $\pm$ 10.6%). There was a significant site by treatment interaction, so analyses of vapor pressure were conducted separately for each site (multivariate repeated measure: site $P < 0.0001$, $F = 58.21$, df = 1, 710; treatment $P < 0.0001$, $F = 15.64$, df = 2, 710; site $\times$ treatment $P = 0.0008$, $F = 7.22$, df = 2, 710). At both sites, vapor pressure was 0.5–1% greater in the mesh bag treatments as compared to controls (Fig. 3; ANOVA: $P < 0.003$, $F > 5.76$ for all), although vapor pressure did not differ between the coarse- and fine-mesh bag treatments. During the warmest part of the day, vapor pressure inside
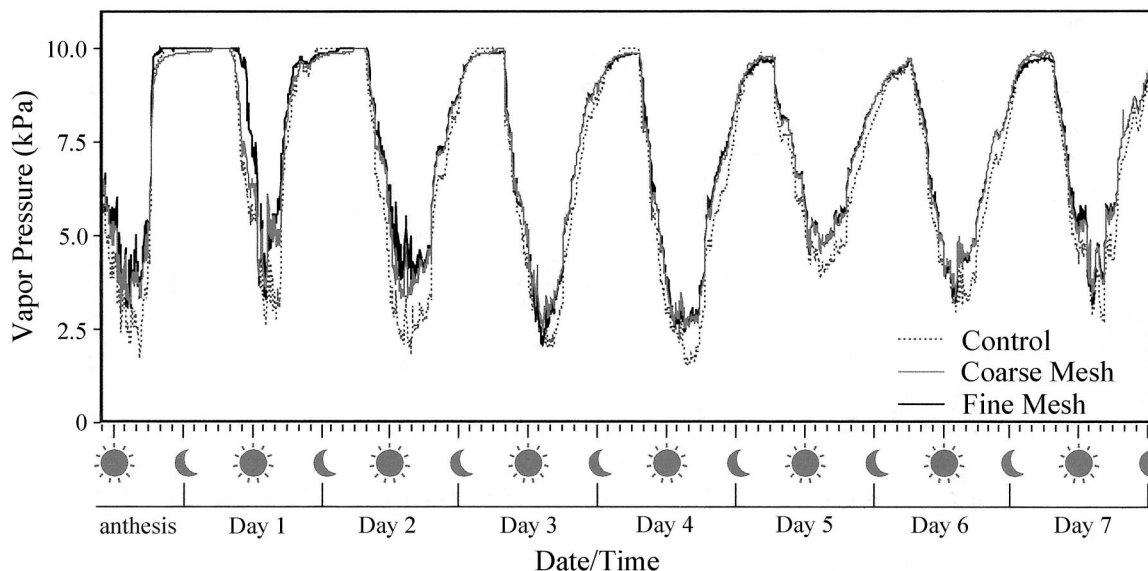
FIG. 3. Example of differences in vapor pressure over time for the three flower treatments. Sun symbols indicate noon, and the moon symbols indicate midnight. Mesh bags and probes were placed on the plant on the day following anthesis.

mesh bags was ~2% greater than vapor pressure immediately outside of control flowers (multivariate repeated measure: $P < 0.004$, $F > 5.80$ for all). Vapor pressure was also 2–3% higher in the mesh bag treatments on days 4, 5, and 6 following anthesis ($P < 0.03$, $\chi^2 > 7.08$, df = 2 for all).

DISCUSSION

Superficial oviposition is the derived condition among *Tegeticula* species and may be an adaptation to escape selective abscission by the host plant (Pellmyr and Leebens-Mack 2000). While the superficial placement of eggs allows *T. cassandra* females to circumvent the mechanism of selective floral abscission, this advantage may come at the cost of increased egg mortality by extrinsic sources. As predicted, *T. cassandra* survivorship was significantly increased in a more favorable microclimate (mesh bags vs. controls), whereas the locule-ovipositing species was unaffected. Survivorship of *T. cassandra* within bagged flowers approached that of *T. yuccasella* within control flowers (mean over all years of study: *T. cassandra* = 27.3% survival vs. *T. yuccasella* = 36.5%). Together these results suggest that the shift to superficial oviposition has exposed moth eggs to specific extrinsic factors of mortality, which are perhaps experienced to a lesser degree by the locule-ovipositing species.

Although other factors may be involved, the most probable mechanism creating this pattern of increased survival in mesh bag treatments is that slight increases in vapor pressure may reduce dehydration of eggs and delay dehydration of the style tissue. The style of *Y. filamentosa* dries out quickly after anthesis and is entirely withered after ~7 d. *Tegeticula cassandra* eggs hatch within 3–6 d of deposition, at which time the

larva burrows through the style tissue and into the locule. The drying of the style tissue may kill eggs and may also contribute to mortality of first-instar larvae by preventing their passage into the locule. A survey of egg and larval mortality found dead, shriveled eggs and larvae within the style tissue (K. A. Segraves, *unpublished data*), suggesting that dehydration may be an important source of mortality. While the present study did not distinguish between mortality at these two life history stages, the same extrinsic factors would be involved. Vapor pressure was significantly increased in mesh bag treatments on days 4, 5, and 6 following anthesis. This time frame corresponds with eclosion of *T. cassandra* eggs and may represent the critical factor explaining the change in survivorship. Egg dehydration is a significant cause of mortality in Lepidoptera and other insect groups (e.g., Neveu et al. 1997, Clark and Faeth 1998, Krasnov et al. 2001, Guarneri et al. 2002, Juliano et al. 2002, Fehrenbach, *in press*). Small changes in relative humidity, such as those detected in this study, can significantly decrease hatching success in other insects (Neveu et al. 1997). If dehydration is causing egg mortality, an increase in vapor pressure during the final stages of egg development may create a less stressful environment for superficially laid eggs. Accordingly, vapor pressure was also increased during the warmest part of the day, another time when conditions may be critical for moth eggs.

Vapor pressure and regional climate also differed somewhat between the two sites. Vapor pressure was significantly lower at Eglin where *T. yuccasella* survivorship was measured. These differences in climate could confound the results; however, if dehydration is an important source of mortality for *T. yuccasella*, survivorship would be predicted to be lower in this rel-

atively dry climate. In contrast, survivorship was the same among treatments and was much higher than *T. cassandra*, even though *T. cassandra* occurs in the climate with higher vapor pressure. An alternative that may also explain the reduction in *T. cassandra* survivorship is egg inviability. Egg viability was not measured directly, although a survey of egg and larval mortality showed that at least 40% of *T. cassandra* eggs hatch to first instar larvae (K. A. Segraves., *unpublished data*), which is a much larger proportion than eggs surviving in the control groups of the present study. This suggests that while egg inviability may be an important source of mortality for *T. cassandra*, there are probably additional factors that also contribute to egg death.

The especially large difference between years in *T. yuccasella* survivorship is most likely a site-specific artifact. In 2001 the fruit at the Eglin site were exceptionally small and misshapen, and fruit abscission rates were extremely high, with a majority of plants losing 100% of their fruit. Moreover, few moth larvae survived at this site and most fruit lacked larvae altogether. Nearby plants within 200 m of the study site were unaffected; they had normal fruit size and shape and had typical levels of larval infestation. The specific causes are unknown, but may be related to poor pollination quality (i.e., high degree of self-pollination), herbivory by *Carpophilus melanopterus* (Coleoptera, Nitidulidae), or plant fungal infection, since many fruit were coated with mildew.

Ant predation appeared to be unimportant for egg survivorship for either moth species. There were no differences in survivorship between control flowers and flowers where ants were excluded. Because ant density was not quantified directly, there is the possibility that the treatment failed to exclude ants or that ant densities were low on control plants. Ants on *Y. filamentosa* tended to search on the inflorescence stalk and side branches, only occasionally searching inside of flowers (K. A. Segraves, *personal observation*). Such foraging behavior may not be conducive to finding moth eggs, or the eggs were simply too small to be energetically worthwhile. Another caveat is that this study only tested for an effect due to predation by crawling insects and arthropods. Placing mesh bags on flowers excludes all predators, and although predation by flying insects was not observed, this may have contributed to mortality in control flowers. I have observed flying predators (reduviids) on yucca inflorescences, but a vast majority of the insects found on *Y. filamentosa* are plant feeders (D. M. Althoff and K. A. Segraves, *unpublished manuscript*).

Anecdotal evidence of egg mortality from another superficially ovipositing yucca moth, *T. superficiella*, indicates a similar response to floral cage treatments (Addicott and Bao 1999). When flowers containing *T. superficiella* eggs were placed into individual flower cages, there was an increase in egg or larval survi-

vorship (Addicott and Bao 1999). Whether *T. superficiella* mortality is caused by predation, parasitism, dehydration, or another factor remains to be tested.

Biotic or abiotic factors extrinsic to the interaction may be quite important in regulating the obligate mutualism between some yuccas and their moths. These factors may significantly decrease egg or larval survival and may translate into a substantial increase in plant reproduction. For example, each *T. cassandra* larva consumes ~15% of the seeds within a fruit, and on average, there are 1.38 ± 0.08 larvae per fruit ($N = 585$ fruit, range 0–11 larvae per fruit). This means an overall seed loss of ~20–22% for a plant pollinated by *T. cassandra* at typical levels of larval survivorship. At least 40% of eggs laid hatch to first-instar larvae, and if all of these survived, seed consumption would increase to 39–42% per plant, a substantial loss of reproduction. Although other factors certainly influence plant reproduction, this difference in seed consumption may have important consequences for the stability of this mutualism.

The present study suggests that factors external to the interaction between *T. cassandra* and *Y. filamentosa* may prevent overexploitation. This, however, does not preclude the possibility of the role of intrinsic factors. For example, floral morphology is known to differ among populations of *Y. filamentosa* (K. A. Segraves and O. Pellmyr, *unpublished data*), and some of the differences in floral traits such as style length may impact larval survivorship. Likewise, *T. yuccasella* may also be regulated by extrinsic factors, although perhaps to a lesser extent than *T. cassandra*. Furthermore, the demonstration of an increase in egg or larval mortality may not necessarily limit moth population sizes. Mortality may be high, but population growth rates could still remain high enough that the mutualism becomes unbalanced. The current levels of seed predation by *T. cassandra* suggest that at least for the present, moth population growth rates are low enough to prevent overexploitation (i.e., ~80% of seeds produced remain intact).

For the obligate pollination mutualism between senita cacti and senita moths, extrinsic factors may also be responsible for regulating the mutualism (Holland and Fleming 1999). In this mutualism, female senita moths actively pollinate and lay an egg on the flower surface, and the larva bores through the corolla and into the top of the developing fruit where it consumes a fraction of the seeds. Unlike the yucca–yucca moth interaction, however, larval damage by senita moths is very high, resulting in certain abscission of the fruit and loss of all seeds. This interaction is mutualistic, because in many instances, the eggs or larvae do not survive and the fruit remains intact. Considerable mortality occurs at the egg stage and may be associated with dehydration or predation (Holland and Fleming 1999). Similarly, parasitoids, fungi, or bacteria may regulate stability in a fly–fungus mutualism by causing

high levels of mortality of fly eggs and larvae (Bultman et al. 2000). These examples highlight the importance of examining extrinsic factors in mutualistic interactions.

While intrinsic regulatory mechanisms remain an interesting and important evolutionary force in the maintenance of mutualisms, the assessment of extrinsic sources is required to further our understanding of the evolution of mutualisms. Because the relative importance of extrinsic factors in a given interaction will depend on the local ecological conditions, the outcome of an interaction may vary both spatially and temporally, emphasizing the need for long-term studies of mutualisms over large geographic scales (Thompson 1994, Thompson and Cunningham 2002).

Literature Cited

Addicott, J. F. 1998. Regulation of mutualism between yuccas and yucca moths: population level processes. Oikos **81**: 119–129.

Addicott, J. F., and T. Bao. 1999. Limiting the costs of mutualism: multiple modes of interaction between yuccas and yucca moths. Proceedings of the Royal Society of London Series B **266**:197–202.

Aker, C. L. 1982. Regulation of flower, fruit and seed production by a monocarpic perennial, *Yucca whipplei*. Journal of Ecology **70**:357–372.

Axelrod, R., and W. D. Hamilton. 1981. The evolution of cooperation. Science **211**:1390–1396.

Axén, A. H., and N. E. Pierce. 1998. Aggregation as a cost-reducing strategy for lycaenid larvae. Behavioral Ecology **9**:109–115.

Breton, L. M., and J. F. Addicott. 1992. Density-dependent mutualism in an aphid–ant interaction. Ecology **73**:2175–2180.

Bronstein, J. L. 1994. Conditional outcomes in mutualistic interactions. Trends in Ecology and Evolution **9**:214–217.

Bull, J. J., and W. R. Rice. 1991. Distinguishing mechanisms for the evolution of cooperation. Journal of Theoretical Biology **149**:63–74.

Bultman, T. L., A. M. Welch, R. A. Boning, and T. I. Bowdish. 2000. The cost of mutualism in a fly–fungus interaction. Oecologia **124**:85–90.

Chauvin, G., and R. Barbier. 1972. Permeability and ultra-structures of eggs of two Lepidoptera: *Monopis rusticella* and *Trichophaga tapetzella*. Journal of Insect Physiology **18**:1447–1462.

Clark, B. R., and S. H. Faeth. 1998. The evolution of egg clustering in butterflies: a test of the egg desiccation hypothesis. Evolutionary Ecology **12**:543–552.

Cushman, J. H., and T. G. Whitham. 1989. Conditional mutualism in a membracid–ant association: temporal, age-specific, and density-dependent effects. Ecology **70**:1040–1047.

Del-Claro, K., and P. S. Oliveira. 2000. Conditional outcomes in a neotropical treehopper–ant association: temporal and species-specific variation in ant protection and homopteran fecundity. Oecologia **124**:156–165.

Denison, R. F. 2000. Legume sanctions and the evolution of symbiotic cooperation by rhizobia. American Naturalist **156**:567–576.

Doebeli, M., and N. Knowlton. 1998. The evolution of interspecific mutualisms. Proceedings of the National Academy of Sciences (USA) **95**:8676–8680.

Fehrenbach, H. *In press*. Eggs. Chapter 18 *in* N. P. Kristensen, editor. Lepidoptera: Handbuch der Zoologie/Handbook of Zoology IV/36. W. de Gruyter, Berlin, Germany.

Force, D. C., and M. L. Thompson. 1984. Parasitoids of the immature stages of several southwestern yucca moths. Southwestern Naturalist **29**:45–56.

Guarneri, A. A., C. Lazzari, L. Diotaiuti, and M. G. Lorenzo. 2002. The effect of relative humidity on the behaviour and development of *Triatoma brasiliensis*. Physiological Entomology **27**:142–147.

Herre, E. A. 1999. Laws governing species interactions? Encouragement and caution from figs and their associates. Pages 209–237 *in* L. Keller, editor. Levels of selection. Princeton University Press, Princeton, New Jersey, USA.

Herre, E. A., N. Knowlton, U. G. Mueller, and S. A. Rehner. 1999. The evolution of mutualisms: exploring the paths between conflict and cooperation. Trends in Ecology and Evolution **14**:49–53.

Herre, E. A., and S. A. West. 1997. Conflict of interest in a mutualism: documenting the elusive fig wasp–seed trade-off. Proceedings of the Royal Society of London Series B **264**:1501–1507.

Hibbett, D. S., L. B. Gilbert, and M. J. Donoghue. 2000. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. Nature **407**:506–508.

Holland, J. N., and D. L. DeAngelis. 2001. Population dynamics and the ecological stability of obligate pollination mutualisms. Oecologia **126**:575–586.

Holland, J. N., and D. L. DeAngelis. 2002. Ecological and evolutionary conditions for fruit abortion to regulate pollinating seed-eaters and increase plant reproduction. Theoretical Population Biology **61**:251–263.

Holland, J. N., D. L. DeAngelis, and J. L. Bronstein. 2002. Population dynamics and mutualism: functional responses of benefits and costs. American Naturalist **159**:231–244.

Holland, J. N., and T. H. Fleming. 1999. Mutualistic interactions between *Upiga virescens* (Pyralidae), a pollinating seed-consumer, and *Lophocereus schottii* (Cactaceae). Ecology **80**:2074–2084.

James, C. D., M. T. Hoffman, D. C. Lightfoot, G. S. Forbes, and W. G. Whitford. 1993. Pollination ecology of *Yucca elata*. Oecologia **93**:512–517.

Johnson, N. C., J. H. Graham, and F. A. Smith. 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. New Phytologist **135**:575–585.

Juliano, S. A., G. F. O'Meara, J. R. Morrill, and M. M. Cutwa. 2002. Desiccation and thermal tolerance of eggs and the coexistence of competing mosquitos. Oecologia **130**:458–469.

Keeley, J. E., S. C. Keeley, C. C. Swift, and J. Lee. 1984. Seed predation due to the yucca-moth symbiosis. American Midland Naturalist **112**:187–191.

Krasnov, B. R., I. S. Khokhlova, L. J. Fielden, and N. V. Burdelova. 2001. Development rates of two *Xenopsylla* flea species in relation to air temperature and humidity. Medical and Veterinary Entomology **15**:249–258.

Marr, D., and O. Pellmyr. 2003. Effect of pollinator inflicted ovule damage on floral abscission in the yucca–yucca moth mutualism: the role of mechanical and chemical factors. Oecologia, *in press*.

Milliken, G. A., and D. E. Johnson. 1992. Analysis of messy data. Volume 1. Designed experiments. Chapman and Hall, New York, New York, USA.

Morales, M. A. 2000. Mechanisms and density dependence of benefit in an ant–membracid mutualism. Ecology **81**: 482–489.

Murray, M. G. 1985. Figs (*Ficus* spp.) and fig wasps (Chalcidoidea, Agaonidae): hypotheses for an ancient symbiosis. Biological Journal of the Linnean Society **26**:69–81.

Nefdt, R. J. C., and S. G. Compton. 1996. Regulation of seed and pollinator production in the fig–fig wasp mutualism. Journal of Animal Ecology **65**:170–182.

Neveu, N., X. Langlet, E. Brunel, M. Lahmer, G. Boivin, M. R. Allo, and J. P. Nénon. 1997. The fine structure of the egg shells of the cabbage maggot, *Delia radicum* L. (Diptera: Anthomyiidae), and its close relation with developmental conditions and oviposition site. Canadian Journal of Zoology **75**:535–541.

Offenberg, J. 2001. Balancing between mutualism and exploitation: the symbiotic interaction between *Lasius* ants and aphids. Behavioral Ecology and Sociobiology **49**:304–310.

Pellmyr, O. 1999. Systematic revision of the yucca moths in the *Tegeticula yuccasella* complex (Lepidoptera: Prodoxidae) north of Mexico. Systematic Entomology **24**:243–271.

Pellmyr, O., and C. J. Huth. 1994. Evolutionary stability of mutualism between yuccas and yucca moths. Nature **372**: 257–260.

Pellmyr, O., and J. Leebens-Mack. 1999. Forty million years of mutualism: evidence for Eocene origin of the yucca–yucca moth association. Proceedings of the National Academy of Sciences (USA) **96**:9178–9183.

Pellmyr, O., and J. Leebens-Mack. 2000. Reversal of mutualism as a mechanism for adaptive radiation in yucca moths. American Naturalist **156**:S62–S76.

Powell, J. A. 1984. Biological interrelationships of moths and *Yucca schotti* (Lepidoptera: Incurvariidae, Cochylidae, Blastobastidae). University of California Publications in Entomology 100.

Richter, K. S., and A. E. Weis. 1995. Differential abortion in the yucca. Nature **376**:557–558.

Riley, C. V. 1873. On a new genus in the lepidopterous family Tineidae, with remarks on the fertilization of *Yucca*. Transactions of the St. Louis Academy of Science **3**:55–64.

Ryan, R. B. 1985. Mortality of eggs of the larch casebearer (Lepidoptera: Coleophoridae) in Oregon. Canadian Entomologist **117**:991–994.

SAS Institute. 1998. JMP 3.2.1. SAS Institute, Cary, North Carolina, USA.

Schwartz, M. W., and J. D. Hoeksema. 1998. Specialization and resource trade: biological markets as a model of mutualisms. Ecology **79**:1029–1038.

Sherratt, T. N., and G. Roberts. 2002. The stability of cooperation involving variable investment. Journal of Theoretical Biology **215**:47–56.

Thompson, J. N. 1994. The coevolutionary process. University of Chicago Press, Chicago, Illinois, USA.

Thompson, J. N., and B. M. Cunningham. 2002. Geographic structure and dynamics of coevolutionary selection. Nature **417**:735–738.

Thompson, J. N., and O. Pellmyr. 1992. Mutualism with pollinating seed parasites amid co-pollinators: constraints on specialization. Ecology **73**:1780–1791.

Trivers, R. L. 1971. The evolution of reciprocal altruism. Quarterly Review of Biology **46**:35–57.

Weiblen, G. D., D. W. Yu, and S. A. West. 2001. Pollination and parasitism in functionally dioecious figs. Proceedings of the Royal Society of London Series B **268**:651–659.

West, S. A., and E. A. Herre. 1994. The ecology of the New World fig-parasitizing wasps *Idarnes* and implications for the evolution of the fig–pollinator mutualism. Proceedings of the Royal Society of London Series B **258**:67–72.

West, S. A., E. T. Kiers, E. L. Simms, and R. F. Denison. 2002. Sanctions and mutualism stability: why do rhizobia fix nitrogen? Proceedings of the Royal Society of London Series B **269**:685–694.

Wilson, R. D., and J. F. Addicott. 1998. Regulation of mutualism between yuccas and yucca moths: is oviposition behavior responsive to selective abscission of flowers? Oikos **81**:109–118.