

ROLES OF FOOD QUALITY AND ENEMY-FREE SPACE IN HOST USE BY A GENERALIST INSECT HERBIVORE

MICHAEL S. SINGER,^{1,4} DANIELA RODRIGUES,² JOHN O. STIREMAN III,³ AND YVES CARRIÈRE¹

¹Department of Entomology and Center for Insect Science, University of Arizona, Tucson, Arizona 85721 USA

²Universidade Federal do Rio Grande do Sul, IB, PPG Biologia Animal, Departamento de Zoologia, Avenida Bento Gonçalves, 9.500, Bloco IV, Prédio 43435, Bairro Agronomia, CEP 91.501-970, Porto Alegre-RS-Brazil

³Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721 USA

Abstract. The relative importance of food quality vs. enemy-free space remains an unresolved but central issue in the evolutionary ecology of host use by phytophagous insects. In this study, we investigate their relative importance in determining host-plant use by a generalist caterpillar, *Estigmene acrea* (Lepidoptera: Arctiidae). In nature, *E. acrea* late-instar caterpillars preferred *Senecio longilobus* (Asteraceae), which contains pyrrolizidine alkaloids that the caterpillars sequester, over *Viguiera dentata* (Asteraceae), a natal host, and typically suffered a 28% mortality risk from parasitoids. We hypothesized that the natural, mixed diet of caterpillars provides high-quality food via hosts like *Viguiera* as well as antiparasitoid defense via sequestered toxins from *Senecio*. We found that a pure *Viguiera* diet provides superior growth performance over a pure *Senecio* or mixed diet in the absence of parasitism. However, when parasitism risk is at least moderate, the mixed diet provides a survival advantage over the pure diets of *Viguiera* or *Senecio*. We therefore conclude that the balance between benefits of growth (food quality) and defense (enemy-free space) maintains the use of a mixed diet in nature. Furthermore, the value of enemy-free space supercedes the value of food quality in determining the host-plant preference of late-instar caterpillars.

Key words: Arctiidae; diet choice; enemy-free space; *Estigmene acrea*; generalist insect herbivore; host preference; parasitoids; polyphagy; *Senecio longilobus*; Tachinidae; tritrophic interactions; *Viguiera dentata*.

INTRODUCTION

Studies of the evolutionary ecology of host-plant use by phytophagous insects have historically approached this topic from two different viewpoints. A bi-trophic view framed the theories of phytochemical coevolution and apparency (sensu Cornell and Hawkins 2003): host-use patterns primarily result from interactions between the herbivores and the chemical or physical characteristics of their host plants (e.g., Dethier 1954, Ehrlich and Raven 1964, Feeny 1976, Berenbaum 1981). Implicit in this influential view is the physiological efficiency hypothesis, that natural selection from host-plant (food) quality (including nutrients and secondary chemistry) drives the evolution of herbivore diet (e.g., Dethier 1954, Scriber and Slansky 1981, Singer 2001). Patterns of host-plant use by phytophagous insects, however, alternatively might result from tri-trophic interactions (e.g., Price et al. 1980, Dyer and Floyd 1993, Dicke 2000). This view invokes the concept of enemy-free space (Jeffries and Lawton 1984), hypothesizing that phytophagous insects use particular host plants in

particular ways to enhance survival via defense or refuge from their natural enemies.

Direct tests of the relative importance of these views via their attendant hypotheses have been few, yet such tests are critical to understanding the evolutionary ecology of host-plant use by phytophagous insects (Denno et al. 1990, Camara 1997a, Lill 2001). It often may be difficult to experimentally disentangle the physiological efficiency and enemy-free space hypotheses for herbivores that gain both nutritional and defensive benefits from a single, or small set of, host-plant species (but see e.g., Denno et al. 1990, Camara 1997a, b, Kessler and Baldwin 2002). An alternative approach is the study of dietary generalist herbivores, for which the great variation in natural diet allows direct experimental tests of the consequences of different diets for herbivore performance with and without the presence of carnivores (Karban and English-Loeb 1997, Oppenheim and Gould 2002, Singer and Stireman 2003). In this study, we take this approach to determine whether bi-trophic or tri-trophic interactions best explain host-plant use by the individually polyphagous saltmarsh caterpillar, *Estigmene acrea* Drury (Lepidoptera: Arctiidae).

Specifically, we tested the hypotheses that individual *E. acrea* caterpillars eat a mixture of plant species either to enhance growth performance (physiological efficiency hypothesis), or to enhance resistance against

Manuscript received 12 December 2003; revised 25 March 2004; accepted 29 March 2004. Corresponding Editor: J. T. Cronin.

⁴ Present address: Department of Biology, Wesleyan University, Hall-Atwater Labs, Room 259, Middletown, Connecticut 06459 USA. E-mail: msinger@wesleyan.edu

their parasitoids (enemy-free space hypothesis). We also tested the following behavioral corollaries of each hypothesis. According to the physiological efficiency hypothesis, caterpillars are expected to prefer the plant species most suitable for growth, whereas the enemy-free space hypothesis predicts greater preference for the most defensive plant species. To address these issues, we assessed (1) caterpillar preference for two key host-plant species in nature, (2) the degree of mortality inflicted by parasitoids in field populations of *E. acrea*, and (3) larval performance on single-species and mixed-species diets of these key plant species, with and without effects of parasitoids on *E. acrea* survival.

STUDY SYSTEM

In southeastern Arizona, USA, *Estigmene acrea* is univoltine, with caterpillars occurring in late summer and early autumn, and frequenting canyons, washes, and riverbanks in association with sunflowers (*Helianthus* and *Viguiera* spp., Asteraceae). The adult moths oviposit clusters of eggs on the mature leaves of sunflowers and, to a lesser extent, other plant species (M. Singer, *personal observation*), upon which larvae feed gregariously as early instars (typically first through third). As mid-instar larvae, most caterpillars leave their natal host plant, wandering widely and feeding on a mixture of different herbaceous and broadleaf woody plant species throughout the duration of development (6–8 instars in total). Among these host plants are two plant species that contain pyrrolizidine alkaloids (PAs): *Senecio longilobus* Benth. (Asteraceae), and *Crotalaria pumila* Ortega (Fabaceae). *Estigmene acrea* caterpillars frequently eat these patchily distributed plant species where and when they occur (M. Singer, *personal observation*), suggesting a preference for them. Collections of late-instar larvae of *E. acrea* in southern Arizona typically yield relatively high frequencies of mortality from parasitoids relative to other caterpillars in the same community (Stireman and Singer 2003a). However, it is not known precisely during which larval stages attack by parasitoids occurs. These field observations, together with laboratory experiments demonstrating that PAs stimulate feeding by *E. acrea* caterpillars (Bernays et al. 2002) and are sequestered by them (Hartmann et al. 2004), suggested that *E. acrea* caterpillars might gain resistance against parasitoids by using the PAs from *Senecio* and *Crotalaria* plants. In addition, studies with two other woolly bear caterpillar species have shown that a caterpillar's likelihood of surviving an attack by parasitoids can be mediated by host plants (Karban and English-Loeb 1997, Singer and Stireman 2003). In the present system, a caterpillar always dies if a parasitoid emerges from it.

METHODS

Host plant preference in the field

We observed a total of 54 individual *Estigmene acrea* final instars under natural conditions during the late

summers of 2001 (Gardner Canyon, details previously discussed), 2002 (Box Canyon, details previously discussed), and 2003 (Santa Rita Experimental Range, Santa Rita Mountains, Pima County, Arizona, USA; Mowry Wash, Patagonia Mountains, Santa Cruz County, Arizona, USA). Each caterpillar was observed continuously over a 6-h period (except four caterpillars that were each observed for 5 h due to the onset of a severe storm). We recorded the duration of resting, walking, and feeding events, as well as all encounters with host plants (contacting a plant with mouthparts). Based on previous work on a related system (Singer and Stireman 2001), we considered two parameters to be particularly sensitive measures of host preference: the probability of accepting a plant species upon encountering it, and the average duration of feeding events if the plant species is accepted.

To assess the probabilities of caterpillars accepting *Viguiera dentata* and *Senecio longilobus*, we used the data in the following way. For each caterpillar, a series of encounters with plant species typically began each time the caterpillar walked away from a plant after feeding on it. Each plant species encountered and rejected (not immediately fed upon) was scored as receiving a single encounter in the series (even if there were repeated encounters with the same species). The series ended when the caterpillar began to feed again and we scored the plant species fed upon as receiving a single encounter and a single acceptance. We used this scoring protocol (recognizing an encounter as the discovery of a new plant species in the series) to reduce the influence of local plant abundance as well as the temporary physiological state of the caterpillar on the calculated acceptability values. When the caterpillar next left the host plant, the scoring process was reiterated until the next feeding event. This process was repeated for the duration of the observation period. For each caterpillar, all of the encounters and acceptances of each plant species over the observational period were summed. The acceptance probability of each plant species was calculated as the summed number of acceptances divided by the summed number of encounters. The average acceptance probabilities of *Viguiera* and *Senecio* were calculated as the means of their acceptance probabilities from all of the individual caterpillars that encountered these plant species. We used a *t* test on angularly transformed acceptance probabilities of individual caterpillars to compare acceptance probabilities of the plant species (SAS Institute 2000).

We calculated the average duration of feeding events on each plant species by computing the mean of the median feeding-bout durations of individual insects that fed on each plant species. Medians were used because bout durations were not normally distributed. In this data set, the sets of caterpillars that encountered and fed on *Viguiera* or *Senecio* consisted of different individuals. We used a *t* test on log-transformed median feeding bout durations of individual caterpillars to

compare average feeding bout durations on the plant species (SAS Institute 2000).

Parasitism in the field

To quantify the mortality risk imposed by parasitoids on *E. acrea* caterpillars, we collected 14 samples of *E. acrea* late-instar caterpillars (total of 544; $N \geq 19$ caterpillars per sample) from 10 different field sites in southeastern Arizona (late summers, 1996–2001). Caterpillars were taken to the laboratory and reared on a wheat-germ-based synthetic diet (Yamamoto 1969) until they died or pupated. All adult parasitoids that emerged were collected and identified. We recorded the frequency of mortality from parasitism of each collection, and quantified the association between the sample size of each collection and the frequency of parasitism, using a simple linear regression (SAS Institute 2000).

Performance experiments

We conducted two parallel experiments to address the physiological efficiency and enemy-free space hypotheses. In each experiment, we reared *E. acrea* caterpillars on each of three different diet treatments: *Viguiera dentata* alone, *Senecio longilobus* alone, and *Viguiera* and *Senecio* in combination. The single-species diets were intended to test the consequences of eating a natal host plant or a highly acceptable non-natal host plant on caterpillar performance with and without interactions with parasitoids. The mixed-species diet more closely resembles the caterpillar's natural diet (M. Singer, unpublished data), and was used to evaluate the feeding choices of caterpillars and their consequences in relation to the other two treatments.

Experiment 1 was conducted in the absence of parasitoids by rearing caterpillars on experimental diets throughout their entire larval development. We obtained *E. acrea* by collecting adult female moths at ultraviolet light (Box Canyon, Santa Rita Mountains, Pima County, Arizona, USA, August 2002). When eggs from two of these females hatched in the laboratory, 20 neonates (10 from each female) were haphazardly assigned to each diet treatment. Each neonate was reared individually in a plastic cup (163 mL) in a walk-in incubator under controlled conditions (25°C, 14:10 L:D photo regime, 40% relative humidity). To mimic their natural life history, we reared caterpillars of all diet treatments solely on *Viguiera* for the first three larval instars. On the day when a caterpillar molted to the fourth instar, it was given its previously assigned experimental diet and was maintained on that diet until it pupated or died. Caterpillars were fed ad libitum and were checked daily. *Viguiera* and *Senecio* shoots were collected from several field sites and were kept refrigerated until use. We measured larval survival, duration, and pupal mass as indices of performance. Duration was calculated as the number of days from hatching until the onset of the prepupal stage (identified by the spinning of a cocoon). We used the mass of pupae that

were freeze-dried to a constant mass. We also determined the sex of each pupa. We used a logistic regression (likelihood ratio test) to analyze larval survival in relation to diet, ANOVA to analyze larval duration and pupal mass in relation to diet, sex, and their interaction, and contrasts to determine differences between treatment means (SAS Institute 2000).

Experiment 2 was conducted in the presence of parasitoids, although we did not manipulate them directly. We collected *E. acrea* caterpillars from nature and transferred them to the laboratory diets. We assumed that some percentage of the caterpillars already would have been attacked by parasitoids. Most caterpillars already had left their natal sunflower hosts by the time of collection, and they varied in their stage of development. We recorded the substrate upon which each was found, and estimated its larval instar based on its size and morphology (median = 5, range 4–7 instars). Caterpillars were collected from three different sites on two different days (Harshaw Creek, Patagonia Mountains, Santa Cruz County, Arizona, USA, 21 August 2002, $N = 12$; Gardner Canyon, Santa Rita Mountains, Pima County, Arizona, USA, 29 August 2002, $N = 75$; Box Canyon, Santa Rita Mountains, Pima County, Arizona, USA, 29 August 2002, $N = 68$). Caterpillars from each site were assigned to each diet treatment in a stratified random way, such that each treatment was represented within each site (totals: *Viguiera*, $N = 52$; *Senecio*, $N = 52$; mixed diet, $N = 51$). Caterpillars were reared in the same way as those in experiment 1. Because these experiments were done concurrently, caterpillars in both experiments received the same set of host-plant material. In experiment 2, we measured only *E. acrea* survival. If mortality occurred, we attributed the cause to either parasitoids (if they emerged) or unknown causes. We also determined the species of all parasitoids that emerged. *Estigmene acrea* pupae were kept for a full year to improve the accuracy of our measures of parasitism frequency. The tachinid *Carcelia languida* frequently diapaused in *E. acrea* pupae, and individual flies emerged in erratic bouts over the following spring and summer. We used a logistic regression (likelihood ratio test) to analyze larval survival in relation to laboratory diet, site of collection, and larval instar at the time of collection, and to analyze parasitoid-caused mortality in relation to laboratory diet (SAS Institute 2000). Because the enemy-free space hypothesis explicitly predicts higher survival on a mixed diet relative to each single-species diet, we used one-tailed tests to test these predictions. We did not include the substrate upon which caterpillars were collected as a factor in these analyses because the numerous levels (substrate types) greatly reduced statistical power while providing limited explanatory power.

To compare the relative fitness effects of enemy-free space and food quality for host-plant use, we used our survival and pupal mass data to calculate estimates of

fitness on each of the three laboratory diets. Assuming that female pupal mass correlates with fecundity, we estimated fitness as the product of larval survival and female pupal mass (Charnov and Shaffer 1973). We used pupal mass values from experiment 1 and survival values from experiment 2 in the bootstrap method with 1000 repetitions to estimate the 95% confidence intervals of fitness on the three diets. For each repetition, the product of the bootstrap estimate of pupal mass and survival was used to evaluate fitness. Pairs of fitness estimates were considered different ($P < 0.05$) when each estimated mean lay outside of the CI for the other mean (Ramsey and Schafer 2002).

RESULTS

Host plant preference in the field

Senecio was more acceptable than *Viguiera*. Final-instar caterpillars in nature accepted *Senecio* ($N = 16$ caterpillars) with a probability of 0.93 ± 0.03 ; (mean ± 1 SE), and *Viguiera* ($N = 26$ caterpillars) with a probability of 0.37 ± 0.08 ; $t = 5.43$, $df = 40$, $P < 0.0001$). In fact, *Senecio*'s acceptability value was the highest among all plant species for which we had data. The difference in feeding bout duration (mean ± 1 SE, in seconds) between plant species was weaker, but in the same direction: for *Senecio*, 174.4 ± 22.2 s ($N = 16$ caterpillars); for *Viguiera*, 118.9 ± 22.9 s ($N = 15$ caterpillars); $t = 2.00$, $df = 29$, $P = 0.056$). All plant species eaten are included in a host-plant species list (Appendix A).

Parasitism in the field

The frequency of mortality from parasitism was $28\% \pm 4\%$ (mean ± 1 SE), with a median of 27% and a range from 6% to 49%. There was a trend for larger collections of caterpillars to have higher percentages of mortality from parasitism ($R^2 = 0.21$, $N = 14$ collections, $P = 0.10$). From these collections, the parasitoid assemblage attacking *Estigmene acrea* in southeastern Arizona consisted of seven species of primary endoparasitoids, four of which are tachinid flies (*Leschenaultia* nr. [near] *adusta*, *Carcelia languida*, *Lespesia aletiae*, *Carcelia reclinata*) accounting for >94% of the parasitism records (Appendix B). We list all plant species eaten by *E. acrea* that were collected for parasitoid sampling (Appendix A).

Performance experiments

In experiment 1, *E. acrea* caterpillars performed better on *Viguiera* than on *Senecio* or the mixed diet. This performance difference was realized through greater pupal mass of *Viguiera* insects relative to the others (Appendix C and Fig. 1; for the contrast V vs. VS diet, $F_{1,30} = 7.234$, $P = 0.012$; for the contrast V vs. S diet, $F_{1,30} = 13.663$, $P = 0.0009$). Female pupae were 35% larger than males ($P = 0.014$), but there was no interaction between sex and diet (Appendix C). There was

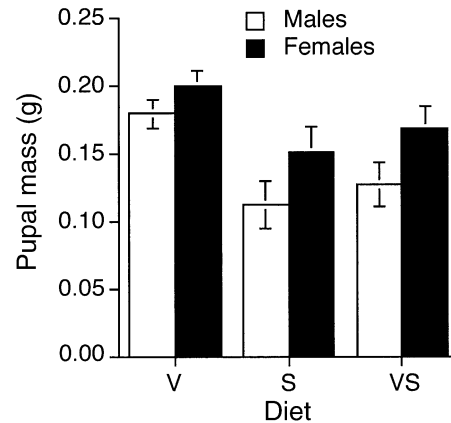


FIG. 1. Mean (± 1 SE) masses of *Estigmene acrea* dry pupae from experiment 1, separated by laboratory diet treatment and sex. Diets V, S, and VS denote *Viguiera dentata*, *Senecio longilobus*, and *Viguiera dentata* + *Senecio longilobus*, respectively. See Appendix C for statistics.

also a trend of better survival on *Viguiera* (logistic regression; likelihood ratio $\chi^2 = 4.87$, $df = 2$, $P = 0.087$) relative to the other diets. Larval duration did not differ among the diet treatments, although female larvae had marginally significantly longer development than males (Appendix C). Daily checks of each caterpillar showed that insects offered the mixed diet did, in fact, spend time on both plant species (data not shown).

By contrast, experiment 2 revealed a significant survival advantage to caterpillars offered the mixed diet when the parasitism frequency was intermediate. An initial analysis shows that both collection site ($\chi^2 = 51.75$, $df = 2$, $P < 0.0001$) and laboratory diet ($\chi^2 = 6.66$, $df = 2$, $P = 0.036$) significantly influenced larval survival, whereas the instar of caterpillars at the time of collection did not ($\chi^2 = 2.48$, $df = 3$, $P = 0.48$). When survival and mortality from parasitoids and other causes are separated by both collection site and laboratory diet (Fig. 2), it is clear that there are different tritrophic outcomes at Gardner and Box Canyons. The Harshaw Canyon sample was too small to analyze alone, so we have included it only in the total values for all canyons combined (Fig. 2). At Box Canyon, where the overall parasitism frequency (35%) was slightly above average, caterpillar survival differed significantly across diet treatments (logistic regression; likelihood ratio $\chi^2 = 9.797$, $df = 2$, $P = 0.0075$). Insects in the mixed treatment had higher survival than those given either *Viguiera* (logistic regression; $\chi^2 = 3.23$, $df = 1$, one-tailed $P = 0.036$) or *Senecio* (logistic regression; $\chi^2 = 9.70$, $df = 1$, one-tailed $P = 0.0018$). As we predicted, parasitoid-caused mortality of caterpillars in the mixed treatment was lower than that of caterpillars in the *Viguiera* treatment (logistic regression; $\chi^2 = 4.53$, $df = 1$, one-tailed $P = 0.017$), but not different than that of caterpillars in the *Senecio* treat-

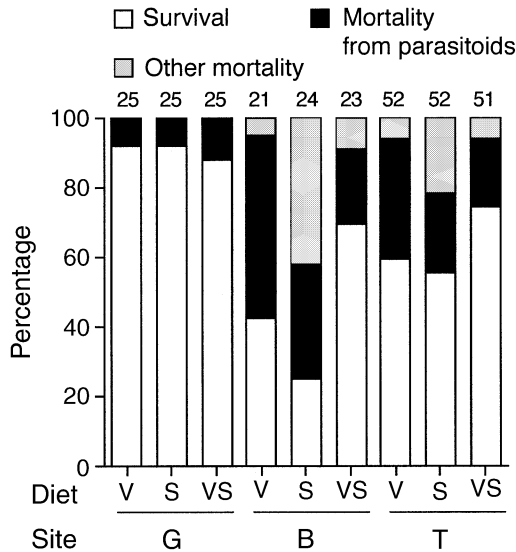


FIG. 2. Percentages of *Estigmene acrea* larval survival, mortality from parasitoids, and mortality from unknown causes ("other mortality") from experiment 2. Data are grouped by field collection site and laboratory diet treatment. Diets V, S, and VS denote *Viguiera dentata*, *Senecio longilobus*, and *Viguiera dentata* + *Senecio longilobus*, respectively. Sites G, B, and T are Gardner Canyon (Santa Rita Mountains), Box Canyon (Santa Rita Mountains), and combined totals for all sites (Gardner, Box, and Harshaw Canyons), respectively. See *Results: performance experiments* for statistics.

ment (logistic regression; $\chi^2 = 0.795$, $df = 1$, $P = 0.37$). The reduced survival of caterpillars given *Senecio* relative to caterpillars given the mixed diet was therefore due to other causes of mortality. In contrast, at Gardner Canyon, where parasitism was low (9%), overall survival (logistic regression; $\chi^2 = 0.304$, $df = 2$, $P = 0.86$), or the likelihood of mortality from parasitoids (the same analysis because all mortality was due to parasitoids) did not differ across diets. Nearly all parasitism was inflicted by the tachinid fly *Carcelia languida*, which appears to be host-specific to *E. acrea* in southeastern Arizona (Stireman and Singer 2003b). The only exception was the parasitism of one caterpillar from Gardner Canyon by the tachinid fly *Leschenaultia* nr. *adusta*.

Fitness estimates

When the parasitism risk was low in nature (e.g., Gardner Canyon in experiment 2), the estimated fitness on *Viguiera*, the mixed diet, and *Senecio* was 0.185 (95% CI, 0.160–0.216), 0.149 (95% CI, 0.102–0.200), and 0.140 (95% CI, 0.130–0.169), respectively. Although estimated fitness on the mixed diet did not differ from that on *Viguiera* and *Senecio*, fitness was significantly greater on *Viguiera* than on *Senecio*. When the apparent frequency of parasitism was slightly above average (e.g., Box Canyon in experiment 2), however, fitness was higher on the mixed diet (0.118; 95% CI, 0.072–0.162) than on *Senecio* (0.038; 95% CI, 0.017–

0.076). Under these conditions, we did not detect a significant difference in fitness between the mixed diet and *Viguiera* (0.086; 95% CI, 0.078–0.168). When we used the survival data combined across sites (26% mortality from parasitism), the results were similar to the Box Canyon results. Fitness was not detectably different between the mixed diet (0.126; 95% CI, 0.082–0.174) and *Viguiera* (0.120; 95% CI, 0.114–0.173), and the latter was superior to *Senecio* (0.085; 95% CI, 0.063–0.115). There was a trend for improved fitness on the mixed diet relative to the pure *Senecio* diet.

DISCUSSION

The results of this study demonstrate the utility of a tri-trophic view of host-plant use by *Estigmene acrea* late-instar caterpillars. The host-plant preference results clearly support the corollary of the enemy-free space hypothesis, that caterpillars should prefer defensively superior plants (e.g., *Senecio longilobus*) over those most suitable for growth (e.g., *Viguiera dentata*).

The exclusively bi-trophic perspective of experiment 1 does not explain why caterpillars customarily choose a mixed diet. In this experiment, a mixed diet was inferior to a pure *Viguiera* diet, causing similar survival and pupal mass as a pure *Senecio* diet. Pupal size generally correlates with fecundity in Lepidoptera (Awmack and Leather 2002), especially in species like *E. acrea* that do not feed during the adult stage. Our finding that there was no growth performance advantage of a mixed diet for *E. acrea* echoes findings from other studies of polyphagous arctiid caterpillars (Bernays and Minkenberg 1997, Hägele and Rowell-Rahier 1999, Singer 2001).

The tri-trophic view framed by the two experiments taken together reveals an ecological trade-off between growth (food quality) and defense (resistance to parasitoids) that can explain why caterpillars eat a mixed diet in nature: to balance demands for growth and defense. This same ecological trade-off has been found to explain the use of multiple host-plant types by other insect herbivores (e.g., Bjorkman et al. 1997, Camara 1997a, b, c, Mira and Bernays 2002). In this case, the mixed diet provided enhanced survival (partly due to antiparasitoid resistance) relative to single-species diets when overall mortality from parasitoids was close to average (Box Canyon, totals for all sites combined). Under these circumstances, estimates of fitness, while not definitive, indicate that this survival advantage can offset the growth performance (and presumed fecundity) cost relative to a pure diet of *Viguiera*, and can also improve fitness over a pure diet of *Senecio*. The survival advantage demonstrated here is likely to underestimate its true magnitude in nature because the sequestration of PAs from *Senecio* is likely to reduce mortality from predators as well as parasitoids (Nishida 2002). By contrast, when parasitism was apparently infrequent (Gardner Canyon), the survival advantage of mixing *Senecio* and *Viguiera* disappeared (Fig. 2).

With such similar survival among diets, we estimated a fitness advantage to eating a pure *Viguiera* diet over a pure *Senecio* diet due to enhanced growth performance (and presumed fecundity) (Fig. 1), with an intermediate fitness on the mixed diet that did not statistically differ from the pure diets. These results indicate that the selective consequence of the single-species and mixed diets depended conditionally on the frequency of attack by parasitoids, thereby changing the relative costs and benefits of antiparasitoid resistance following attack. It is not known if the frequency of attack by parasitoids in this system is dependent on the host plant, although this may be a generally important determinant of enemy-free space for insect herbivores (Barbosa et al. 2001, Lill et al. 2002).

The mechanisms conferring resistance to parasitoids and performance costs in *Senecio*-feeding caterpillars deserve further investigation. We suspect that pyrrolizidine alkaloids (PAs), possessed by *Senecio longilobus*, are involved in the enhanced parasitoid resistance gained from diets containing *Senecio*. There is indirect evidence for this mechanism in the related arctiid, *Grammia geneura* (M. Singer, Y. Carrière, C. Theuring, and T. Hartmann, *unpublished manuscript*). Like *Grammia*, *Estigmene* caterpillars sequester PAs in various body tissues, including the hemolymph (T. Hartmann, *unpublished data*), where larval tachinid parasitoids reside and feed (Clausen 1940). PAs may be directly toxic to parasitoids or they may primarily deter feeding, perhaps causing increased parasitoid locomotion, and enhancing the efficacy of the caterpillar immune system. It is also possible that PAs in *Senecio longilobus* reduce *Estigmene* survival and pupal mass, although these effects could also be due to other characteristics of *Senecio*.

More generally, the results of this study underscore the importance of adopting a tri-trophic perspective in understanding the evolutionary ecology of host-plant use by phytophagous insects (Bernays 1998). This view allows for the conceptual and methodological inclusion of both the physiological efficiency and enemy-free space hypotheses. This is important because food quality's sole consequences for herbivore performance frequently have been used to address the evolution of host specificity (e.g., reviews in Jaenike 1990, Price 1997) and dietary generalism (e.g., Waldbauer and Friedman 1991, Cornell and Hawkins 2003). As Cornell and Hawkins (2003) point out, the explanatory power of such a bi-trophic view of plant-insect coevolution may be limited. Despite the recognition of the possible importance of tri-trophic interactions as selective forces in host-plant use (e.g., Price et al. 1980) for at least two decades, there has been limited empirical work addressing them (Dicke 2000), particularly in relation to macroecological and macroevolutionary patterns (Lill et al. 2002). Nevertheless, recent studies (e.g., Damman 1987, Ohsaki and Sato 1994, Abrahamson and Weis 1997, Kessler and Baldwin 2002), in addition

to this one, provide some mechanistic support for the notion that tri-trophic interactions play a key role in explaining larger scale associations between plants and phytophagous insects (Lill et al. 2002).

ACKNOWLEDGMENTS

Thanks to E. A. Bernays, D. M. Higginson, B. DeGain, and T. Hartmann for assistance with field observations. Plant species were determined with critical assistance from the University of Arizona Herbarium. We also thank Jim Cronin and two anonymous reviewers for their constructive comments on the manuscript. M. S. Singer was funded by the Center for Insect Science (University of Arizona) through NIH Training Grant # 1 K12 Gm00708. D. Rodrigues was supported by a grant from CAPES Foundation/Brazil. J. O. Stireman, Jr. was funded by NSF Dissertation Improvement Grant Number DEB-9801537. Y. Carrière was funded by USDA Biotechnology Risk Assessment Research Grant 2003-04371, USDA NRI Grant 01-35302-09976, and EPA Cooperative Agreement X-82974701-0.

LITERATURE CITED

- Abrahamson, W. G., and A. E. Weis. 1997. Evolutionary ecology across three trophic levels. Princeton University Press, Princeton, New Jersey, USA.
- Awmack, C. S., and S. R. Leather. 2002. Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology* **47**:817–844.
- Barbosa, P. B., et al. 2001. Differential parasitism of macrolepidopteran herbivores on two deciduous tree species. *Ecology* **82**:698–704.
- Berenbaum, M. 1981. Patterns of furanocoumarin distribution and insect herbivory in the Umbelliferae: plant chemistry and community structure. *Ecology* **62**:1254–1266.
- Bernays, E. A. 1998. Evolution of feeding behavior in insect herbivores—success seen as different ways to eat without being eaten. *BioScience* **48**:35–44.
- Bernays, E. A., R. F. Chapman, and T. Hartmann. 2002. A taste receptor neurone dedicated to the perception of pyrrolizidine alkaloids in the medial galeal sensillum of two polyphagous arctiid caterpillars. *Physiological Entomology* **27**:312–321.
- Bernays, E. A., and O. P. J. M. Minkenberg. 1997. Insect herbivores: different reasons for being a generalist. *Ecology* **78**:1157–1169.
- Bjorkman, C., S. Larsson, and R. Bommarco. 1997. Oviposition preferences in pine sawflies: a trade-off between larval growth and defence against natural enemies. *Oikos* **79**:45–52.
- Camara, M. D. 1997a. A recent host range expansion in *Junonia coenia* Hübner (Nymphalidae): oviposition preference, survival, growth, and chemical defense. *Evolution* **51**:873–884.
- Camara, M. D. 1997b. Physiological mechanisms underlying the costs of chemical defence in *Junonia coenia* Hübner (Nymphalidae): a gravimetric and quantitative genetic analysis. *Evolutionary Ecology* **11**:451–469.
- Camara, M. D. 1997c. Predator responses to sequestered plant toxins in buckeye caterpillars: are tritrophic interactions locally variable? *Journal of Chemical Ecology* **23**:2093–2106.
- Charnov, E. L., and W. M. Schaffer. 1973. Life-history consequences of natural selection: Cole's result revisited. *American Naturalist* **107**:791–792.
- Clausen, C. P. 1940. *Entomophagous insects*. Hafner, New York, New York, USA.
- Cornell, H. V., and B. A. Hawkins. 2003. Herbivore responses to plant secondary compounds: a test of phytochemical coevolution theory. *American Naturalist* **161**:507–522.

- Damman, H. 1987. Leaf quality and enemy avoidance by larvae of a pyralid moth. *Ecology* **68**:87–97.
- Denno, R. F., S. Larsson, and K. L. Olmstead. 1990. Role of enemy-free space and plant quality in host-plant selection by willow beetles. *Ecology* **71**:124–137.
- Dethier, V. G. 1954. Evolution of feeding preferences in phytophagous insects. *Evolution* **8**:33–54.
- Dicke, M. 2000. Chemical ecology of host-plant selection by herbivorous arthropods: a multitrophic perspective. *Biochemical Systematics and Ecology* **28**:601–617.
- Dyer, L. A., and T. Floyd. 1993. Determinants of predation on phytophagous insects: the importance of diet breadth. *Oecologia* **96**:575–582.
- Ehrlich, P. R., and P. H. Raven. 1964. Butterflies and plants: a study in coevolution. *Evolution* **18**:586–608.
- Feeny, P. 1976. Plant apparency and chemical defense. *Recent Advances in Phytochemistry* **10**:1–40.
- Hägele, B. F., and M. Rowell-Rahier. 1999. Dietary mixing in three generalist herbivores: nutrient complementation or toxin dilution? *Oecologia* **119**:521–533.
- Hartmann, T., C. Theuring, T. Beuerle, L. Ernst, M. S. Singer, and E. A. Bernays. 2004. Acquired and partially de novo synthesized pyrrolizidine alkaloids in two polyphagous arctiids and the alkaloid profiles of their larval food-plants. *Journal of Chemical Ecology* **30**:229–254.
- Jaenike, J. 1990. Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics* **21**:243–273.
- Jeffries, M. J., and J. H. Lawton. 1984. Enemy free space and the structure of ecological communities. *Biological Journal of the Linnean Society* **23**:269–286.
- Karban, R., and G. English-Loeb. 1997. Tachinid parasitoids affect host plant choice by caterpillars to increase caterpillar survival. *Ecology* **78**:603–611.
- Kessler, A., and I. T. Baldwin. 2002. *Manduca quinquemaculata*'s optimization of intra-plant oviposition to predation, food quality, and thermal constraints. *Ecology* **83**:2346–2354.
- Lill, J. T. 2001. Selection on herbivore life-history traits by the first and third trophic levels: the devil and the deep blue sea revisited. *Evolution* **55**:2236–2247.
- Lill, J. T., R. J. Marquis, and R. E. Ricklefs. 2002. Host plants influence parasitism of forest caterpillars. *Nature* **417**:170–173.
- Mira, A., and E. A. Bernays. 2002. Trade-offs in host use by *Manduca sexta*: plant characters vs. natural enemies. *Oikos* **97**:387–397.
- Nishida, R. 2002. Sequestration of defensive substances from plants by Lepidoptera. *Annual Review of Entomology* **47**:57–92.
- Ohsaki, N., and Y. Sato. 1994. Food plant choice of *Pieris* butterflies as a trade-off between parasitoid avoidance and quality of plants. *Ecology* **75**:59–68.
- Oppenheim, S. J., and F. Gould. 2002. Behavioral adaptations increase the value of enemy-free space for *Heliothis subflexa*, a specialist herbivore. *Evolution* **56**:679–689.
- Price, P. W. 1997. *Insect ecology*. Wiley, New York, New York, USA.
- Price, P. W., C. E. Bouton, P. Gross, B. A. McPherson, J. N. Thompson, and A. E. Weis. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics* **11**:41–65.
- Ramsey, F. L., and D. W. Shafer. 2002. *The statistical sleuth: a course in methods and data analysis*. Duxbury Press, New York, New York, USA.
- SAS Institute. 2000. *JMP version 4. 0*. SAS Institute, Cary, North Carolina, USA.
- Scriber, J. M., and F. Slansky. 1981. The nutritional ecology of immature insects. *Annual Review of Entomology* **26**:183–211.
- Singer, M. S. 2001. Determinants of polyphagy by a woolly bear caterpillar: a test of the physiological efficiency hypothesis. *Oikos* **93**:194–204.
- Singer, M. S., and J. O. Stireman III. 2001. How foraging tactics determine host-plant use by a polyphagous caterpillar. *Oecologia* **129**:98–105.
- Singer, M. S., and J. O. Stireman III. 2003. Does anti-parasitoid defense explain host-plant selection by a polyphagous caterpillar? *Oikos* **100**:554–562.
- Stireman, J. O., III, and M. S. Singer. 2003a. Determinants of parasitoid-host associations: insights from a natural tachinid-lepidopteran community. *Ecology* **84**:296–310.
- Stireman, J. O., and M. S. Singer. 2003b. What determines host range in parasitoids? An analysis of a tachinid parasitoid community. *Oecologia* **135**:629–638.
- Waldbauer, G. P., and S. Friedman. 1991. Self-selection of optimal diets by insects. *Annual Review of Entomology* **36**:43–63.
- Yamamoto, R. T. 1969. Mass rearing of the tobacco hornworm II. Larval rearing and pupation. *Journal of Economic Entomology* **62**:1427–1431.

APPENDIX A

A table showing plant species eaten by *Estigmene acrea* caterpillars during field collections to assess parasitism, and during behavioral observations, is available in ESA's Electronic Data Archive: *Ecological Archives* E085-088-A1.

APPENDIX B

A list of parasitoid species recovered from collections of *E. acrea* (1996–2001) in southeastern Arizona is available in ESA's Electronic Data Archive: *Ecological Archives* E085-088-A2.

APPENDIX C

An ANOVA table showing the effects of diet, sex, and their interaction on performance of surviving insects in experiment 1 is available in ESA's Electronic Data Archive: *Ecological Archives* E085-088-A3.