TRITROPHIC RESPONSES TO SHADING
OF *PASSIFLORA BIFLORA*, A NEOTROPICAL VINE

by

GORDON CASTER WARD

(Under the direction of Chris J. Peterson)

ABSTRACT

One of the major goals of ecology is to understand the relative importance of the abiotic and biotic environment in determining the distribution and abundance of organisms. For terrestrial plants, it is now generally recognized that both bottom-up effects of the abiotic environment and top-down effects of herbivores and their natural enemies can have important consequences. Much less is understood, however, about how these species interactions are altered under different environmental conditions.

In this dissertation, I studied the direct and indirect effects of light availability on the performance of a Neotropical vine with extrafloral nectaries (*Passiflora biflora*). In one experiment, groups of plants were placed in sunny and shaded areas at a field site in Costa Rica, whereas in a second experiment, light availability was manipulated using three kinds of shade huts (0%, 50%, and 90% shade). By selectively permitting or excluding phytophagous insects and/or ants foraging for extrafloral nectar, I evaluated the strength of these interactions and how their relative importance was altered by shading. In both studies, the direct negative effects of shading and herbivores were clearly seen, but the substantial impact of herbivores was little influenced by light availability. Shading also had little effect on ant visitation, and ants were shown to be ineffective plant bodyguards. Parasitoid and predaceous wasps were also observed visiting extrafloral nectaries; they were little influenced by shading, but unlike ants, wasps were effective defenders against heliconiine caterpillars. Taken together, these experiments found little evidence for indirect effects of shading on plant performance or that the relative importance of direct vs. indirect effects was altered by light availability.

For plants in general, I then review what is known about variation in extrafloral nectaries and bodyguard defense, and I organize these prior observations into a general framework for understanding their evolution and phenotypic variation. This framework is defined by three axes of environmental heterogeneity: availability of excess plant resources, risk of plant damage by herbivores, and effectiveness of available plant bodyguards. I conclude by considering a number of ecological and evolutionary predictions that are derived from the proposed framework and suggest several directions for future research.

INDEX WORDS: Alticinae, Ants, Bodyguard protection, Costa Rica, Extrafloral nectaries, Heliconiinae, Herbivory, Light environment, Parasitoids, *Passiflora biflora*, Phenotypic plasticity, Shading, Tropical premontane rain forest, Wasps

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GORDON CASTER WARD

B.S., Beloit College, 1985

M.A., University of Kansas, 1994

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GORDON CASTER WARD

Approved:

Major Professor:	Chris J. Peterson
Committee:	Lisa A. Donovan James L. Hamrick Mark D. Hunter Robert Wyatt

Electronic Version Approved:

Gordhan L. Patel
Dean of the Graduate School
The University of Georgia
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Field research reported here was carried out at the OTS Las Cruces Biological Station in southern Costa Rica. Luis Diego Gómez, the director of the Las Cruces Biological Station, kindly permitted me to carry out my experiments within the grounds of the associated Wilson Botanical Garden. I can only hope that my many scattered plants and shade "huts" contributed in some small way to the Garden's botanical mysteries. I am also greatly indebted to the other Las Cruces staff for their ready assistance and warm friendship throughout the many months I worked there, especially during those long off-season periods when no other guests were present.

During much of my research at Las Cruces, I employed local Costa Rican assistants to help me with my research. The two individuals who worked for me the longest were Sergio and Freddy Ilama, and their tireless efforts and attention to detail were invaluable to the success of my fieldwork. I thank them for being a pleasure to work with and for putting up with my many inexplicable demands and frequently muddy Spanish. I also thank the people of the nearby towns of Copal and San Vito for their support and friendship; in particular, I thank the San Vito shopkeepers for their generous assistance on my many quests for odd equipment and supplies.

Besides the Costa Ricans mentioned above, I also met and became friends with many students, tourists, and fellow researchers who visited the Las Cruces Biological Station while I was there. Many of these people provided invaluable advice and encouragement during critical stages of my research. In particular, I thank the following individuals: Laura Aldrich-Wolfe, Jenny Cruse-Sanders, Peter Curtis, Gretchen Daily, Paul Ehrlich, Larry Gilbert, Sophia Kuo, Christina McCain, Terry McGlynn, Debra Murray, Ronal Murrillo, Tania Rehse, Satya Rhodes-Conway, Taylor Ricketts, Raúl Rojas, Martha Rosemeyer, Dory Ruderfer, Catherine Simpson-Bainbridge, and Brian Spitzer.

Before starting my research in Costa Rica, I had little experience with the collection and identification of insects and other invertebrates, and therefore I relied heavily on the help of many expert biologists for this component of my dissertation. In particular, I thank Cecil Smith (UGA) for his early guidance on methods of insect collection and preservation; Terry McGlynn (USD) for a lucid introduction to the ants; Herman Vega for permission to visit the insect collection at the *Museo Nacional de Costa Rica*; Angela Mora, Elena Ulate, and Ronald Zúñiga (all at INBio, the *Instituto Nacional de Biodiversidad*) for identification of wasps and beetles; Alejandro Valerio (INBio, UIUC) for identification of Braconidae; Isidro Chacón (INBio) for identification of butterflies; Mario Camacho (INBio) for identification of leaf miners; Zaidett Barrientos (INBio) for identification of slugs; Randall Gutiérrez (INBio) for entry of specimen information into the INBio Atta system; Carlos Hernandez (INBio) for taking the time to teach me how to mount beetles like a pro; and Carmen Flores for her invaluable assistance throughout my work at INBio, for which I am forever indebted.

When my dissertation research was complete, I was very pleased to receive permission to deposit my insect and plant specimens in the INBio museum in Santo Domingo de Heredia, Costa Rica. I thank Bernardo Espinoza for accepting my Lepidoptera specimens; Carolina Godoy for accepting my Hymenoptera specimens; Jose Gonzales for accepting my *Passiflora biflora* specimens; and Angel Solis for accepting my Coleoptera specimens. In addition, I must acknowledge Tania Bermúdez and her family for opening their home to me when I worked at INBio and for storing my specimens between visits to Costa Rica.

Although the field experiments reported in this dissertation were conducted at the Las Cruces Biological Station, I maintained throughout my studies a collection of native and tropical *Passiflora* at the UGA Botany Greenhouses, and on several occasions, I occupied a great deal of greenhouse space with elaborate research structures. (Remember those various indoor and outdoor shade cages, and the bubbling hydroponic propagation basins?) In the end, however, this work never developed into another dissertation chapter. Nevertheless, my use of the UGA greenhouses was invaluable to my dissertation research because it permitted me to work out many methodological issues that I later faced in Costa Rica and to explore a variety of tangential research topics, some of which I may pursue in the future. Thus, I feel quite indebted to the staff of the Greenhouses, for their vigilant care of my many passion vines, for the numerous brainstorming sessions on project ideas and methods, and for their friendship and support throughout my time at UGA. Specifically, I thank Andy Tull, Mark Zimmerman, Michael Boyd, and most importantly, Melanie Smith, my onetime neighbor and favorite cheerleader. In addition, I thank Ron Determan of the Atlanta Botanical Garden for giving me the initial cuttings of the tropical species and John MacDougal of the Missouri Botanical Garden for his long-distance assistance with species identification.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW:

UNTANGLING THE ENTANGLED BANK

> *It is interesting to contemplate an entangled bank, clothed with many plants of many kinds, with birds singing on the bushes, with various insects flitting about, and with worms crawling through the damp earth, and to reflect that these elaborately constructed forms, so different from each other, and dependent on each other in so complex a manner, have all been produced by laws acting around us.*

This passage is taken from the very last, and probably most famous, paragraph in the *Origin of Species* (Darwin 1859 [1964], p. 489). Here Darwin uses the phrase "an entangled bank" as a metaphor for what he called the "web of complex relations" that exists among species. As illustrated by this passage, it is clear that Darwin was very aware of the potential complexity of species interactions and that the diversity of species cannot make sense without an understanding of these interactions (Thompson 1994).

Darwin provides several examples of complex interaction webs in the *Origin of Species*. One of the most interesting is his speculation about the importance of cats in the English countryside (Figure 1.1). In this example, he first notes that "humble-bees" (i.e., bumblebees) are indispensable to the fertilization of red clover because the flowers are not visited by other bee species. Based on this observation, he predicts that if humble-bees were to go extinct, the red clover would also. He then notes that the number of humble-bees depends to a great degree on the abundance of field mice because the mice destroy the nests where the bees live, and the number of mice, as everyone knows, largely depends on the number of cats. Hence, as Darwin points out, the presence of cats may determine the frequency of red clover in the English countryside, via their negative direct effect on mice, which results in a positive indirect effect on humble-bees, and therefore a positive indirect effect on red clover.

Other scientists later extended this particular web of Darwin's to include a few additional interactions (Figure 1.1; Vandermeer *et al.* 1985). For instance, it was noted that because red clover provides important feed for cattle and that canned beef provides critical nourishment for sailors on long voyages, the health of the British navy may be ultimately determined by the beneficial effect of cats on red clover. Then in a more humorous vein, Thomas Huxley pointed out the need for consideration of "spinsters" because these women are known to be the great protectors of cats (Vandermeer *et al.* 1985). Therefore, as he concluded, the strength of the British Empire may actually rest on the shoulders of these scorned women.

Although speculative, this classic interaction web is useful to illustrate several key concepts relevant to my dissertation research. First, this web provides a good example of *direct* and *indirect effects* among trophic levels (shown as solid and dashed lines, respectively, in the figures), and second, it emphasizes the observation that all species may interact via both direct and indirect effects, sometimes positively and sometimes negatively.

Darwin's web also shows how long scientists have recognized the potential importance of indirect species interactions. But despite this long recognition, most ecological studies have investigated only direct environmental effects on single species or only the direct effects of one species on another (Thompson 1994). Although usually unstated, such studies assume that these individual pair-wise interactions can be joined together to provide a complete understanding of the entire system. There is no question that many direct effects are strong, meaning that they explain much of the observed variation found in nature, but in most biological communities, it is still largely unknown to what extent the strength or even the direction of these effects is altered by the presence of other species (Strauss 1991, Menge 1995). In other words, the indirect effects of cats or spinsters are only rarely considered or quantified.

Therefore, a major challenge in modern ecology is to determine the relative importance of direct and indirect effects (Strauss 1991, Schoener 1993, Menge 1995, Abrams *et al*. 1996). Are direct effects always stronger than indirect effects, or are indirect effects as strong, or sometimes stronger, than direct effects (Figure 1.2)? An additional challenge is to understand how environmental variation alters the relative importance of these interactions (Oksanen *et al.* 1981, Hunter and Price 1992). For example, does greater environmental productivity have little effect on their relative importance, or do indirect effects increase in strength with greater environmental productivity (Figure 1.3)? Because so little is known about the frequency, magnitude, and importance of indirect effects, their potential ecological impact is often ignored and assumed to be weak or unimportant (Yodzis 1988).

Research objectives

For this dissertation, I studied the tritrophic responses to shading of *Passiflora biflora*, a Neotropical vine with extrafloral nectaries (EFNs), at a premontane field site in southern Costa Rica (Figure 1.4). My research goals were to determine how shading directly affects plant performance, how plant performance is influenced by interactions with insect herbivores and their natural enemies (e.g., ants and wasps), and whether the impact of herbivores and natural enemies is indirectly altered by variation in the plant's light environment. By attempting such a synoptic field study, in which measurements were taken on plants, herbivores, and natural enemies, I have tried to untangle one small corner of Darwin's entangled bank.

Overview of methods

I selected *Passiflora biflora* (Passifloraceae) as the focal species for my research because: (1) it tends to grow in sunny environments, ranging from old fields to successional forest; (2) it is attacked by a variety of insect herbivores, many of which have been well studied (Benson *et al.* 1975, Benson 1978, Gilbert and Smiley 1978, Brown 1981,Gilbert 1991); and (3) it has numerous leaf extrafloral nectaries, which are known to attract ants (Smiley 1978, Apple and Feener 2001) that may then defend the plant against insect herbivores (*sensu* Bentley 1977). I therefore expected to find strong effects of shading, herbivores, and ant bodyguards on plant performance (Figure 1.4).

I conducted my field research at the Wilson Botanical Garden, which is on the Pacific slope of Costa Rica, near the Panamanian border, at about 1100 m elevation. The landscape at this locality consists of a heterogeneous mixture of primary forest fragments, successional forest, and pasture. The Botanical Garden was selected as a study site because it represented a reasonable compromise between light availability, biological realism, experimental control, and site security. As I discovered, however, this site may have been inappropriate for the study of bodyguard defense by ants because ants were uncommon plant visitors at this elevation. But it did prove useful for detection of the important role of wasp bodyguards.

Chapter 2: A synoptic field experiment

The light environment is known to be a very heterogeneous resource in forest communities (Canham *et al.* 1990), and its availability is often a major limitation to plant growth, reproduction, and survival (Denslow 1980, Nicotra *et al.* 1999). It is also known that light availability has diverse direct effects on plant performance, with some species growing best in full sun and others doing best at lower light levels (Mulkey *et al*. 1996). But *why* does plant performance differ between sun and shade? This may seem a simple question, but it is not because plant performance can be a function of both direct and indirect effects. By far, the direct effect of light on photosynthesis and growth has received the most study (Brokaw and Busing 2000), but indirectly, light availability may also alter (1) herbivore attack (Louda and Rodman 1996) or (2) plant protection by the natural enemies of the herbivores (Bentley 1976, de la Fuente and Marquis 1999). Because these different effects of light are not mutually exclusive, each may explain a portion of observed variation in plant performance. Thus, the question is, not "What are the direct effects of light availability on plant performance?" but rather "What is the relative importance of direct and indirect effects?" (Welden and Slauson 1986, Hunter and Price 1992). The latter allows for the existence of multiple interacting forces, without assuming the preeminence of direct effects, and it emphasizes that knowledge of the intensity of an effect may not be sufficient to understand community structure.

In Chapter 2 of this dissertation, I report on an experiment designed to examine the relative importance of direct and indirect effects of shading on *P. biflora*. In this study, groups of plants were placed in sunny and shaded areas within the Botanical Garden, and insect herbivores and/or foraging ants were experimentally permitted or excluded. For the next several months, I observed the effects of the shading and exclusion treatments on plant growth and reproduction to determine the relative impact of the different interactions. Using ANOVA and partial eta-squared values, I then compared the amount of variability associated with each interaction to examine the relative importance of the direct and indirect effects (*sensu* Welden and Slauson 1986, Dyer and Letourneau 1999).

Chapter 3: A closer examination

In Chapter 3, I report on a similar experiment, again using *P. biflora*, but in which the light environment was manipulated using three kinds of shade huts. This method allowed better control of the effects of shading, thereby enabling me to focus on the extent to which variation in bodyguard protection of the plants is caused by variation in light availability. This bodyguard role of ants attracted to plants with EFNs has been well documented, but the degree of plant benefit is highly variable, both within and among species and across environments (Bronstein 1998, de la Fuente and Marquis 1999). Some of this variation in benefit may be explained by the availability of ant bodyguards or by their effectiveness, or by the degree of herbivore attack, but it is largely unknown to what extent bodyguard protection is altered by the abiotic conditions of the plant's environment (Bronstein 1998). Light availability, in particular, may have strong effects on extrafloral nectar production, either at the plant, leaf, or nectary level, because it is known to have strong effects on floral nectar (e.g., Michaud 1990, Rathcke 1992, Boose 1997). Therefore, I predicted that the observed variation in plant performance across different levels of light availability would be due, in part, to the indirect effects of light on bodyguard protection. Clearly, variation in plant performance across light levels may be a function of environmental effects unrelated to bodyguard defense, but the focus of this experiment was on the contribution of differential plant protection by bodyguards. As I found in the companion study of *P. biflora* (Chapter 2), ants were uncommon plant visitors and ineffective plant protectors. Therefore, I addressed in this study the influence of light availability on bodyguard defense in general, including both ant and non-ant bodyguards.

Chapter 4: A general framework

In Chapter 4, I review some of the patterns seen in the distribution, abundance, and behavior of extrafloral nectaries, and attempt to develop a general framework to explain the observed and predicted variation. Although it has long been recognized that the light and soil nutrient environment can affect the functioning of nectaries, most biologists have continued to treat these organs as static life history traits. I believe that there is much opportunity for research on the evolutionary patterns and phenotypic plasticity of extrafloral nectaries, and I have highlighted in this chapter some of the possible directions for future investigation.

Chapter 5: Conclusions

Finally, in Chapter 5, I briefly summarize the major conclusions from my dissertation research and attempt to tie the results together. Potential opportunities for future research are also discussed.

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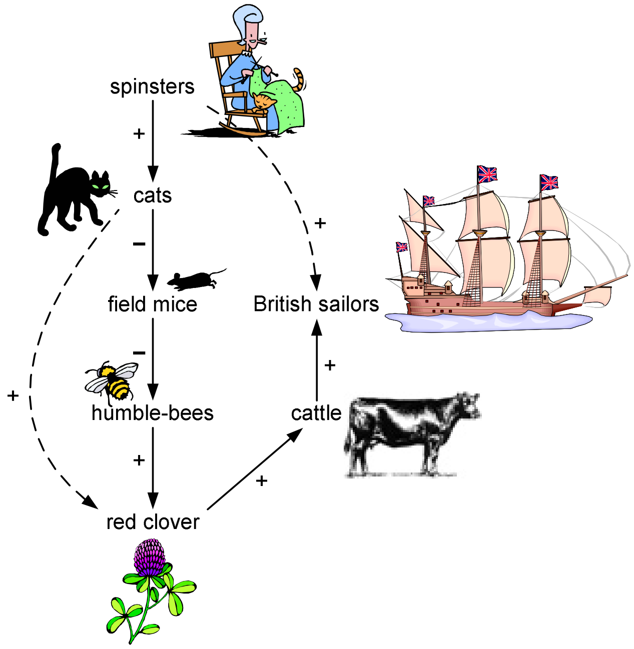

Figure 1.1. Charles Darwin's predictions regarding the effects of cats on red clover, shown with additional interactions suggested by other scientists (Vandermeer *et al.* 1985). Solid lines indicate direct effects; dashed lines indicate indirect effects. See text for details.

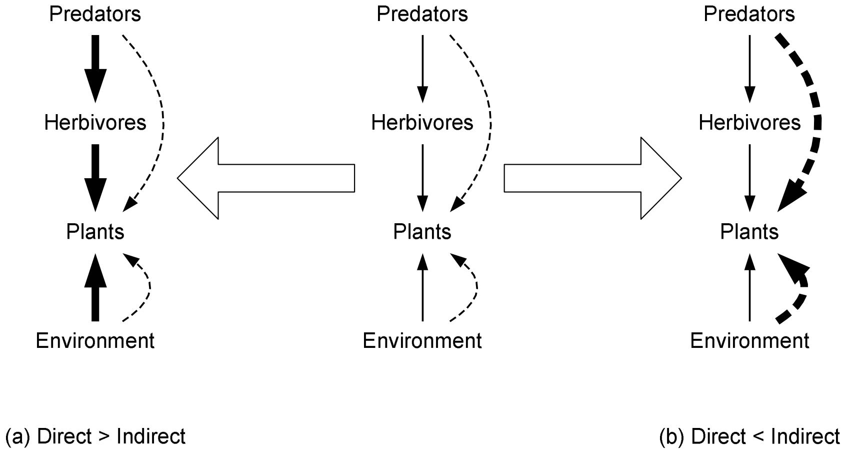

Figure 1.2. What is the relative importance of direct and indirect effects? In (a), direct effects are stronger than indirect effects, whereas in (b), indirect effects are stronger. For simplicity, the indirect effect of the environment on plants is summarized by a single dashed arrow, and potential direct and indirect effects of the environment on herbivores and predators are not shown.

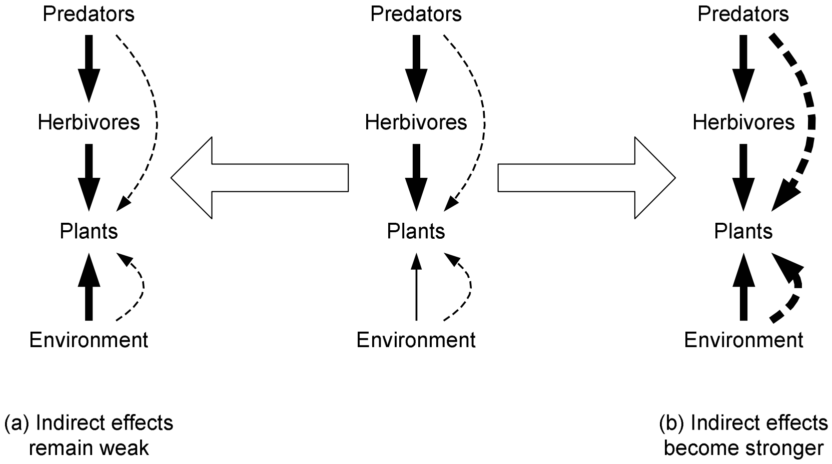

Figure 1.3. How is the relative importance of direct and indirect effects influenced by environmental conditions? In (a), the relative interaction strength is not changed by increasing environmental productivity (e.g., greater sun exposure), whereas in (b), the indirect effects increase in strength.

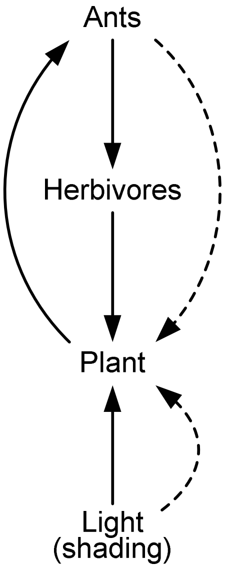

Figure 1.4. Predicted direct and indirect effects of shading on the tritrophic study system. For simplicity, the indirect effect of shading on plants is summarized by a single dashed arrow.

CHAPTER 2

TRITROPHIC RESPONSES TO SHADING OF *PASSIFLORA BIFLORA*, A NEOTROPICAL VINE: THE RELATIVE IMPORTANCE OF DIRECT AND INDIRECT EFFECTS[1]

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[1] Ward, G. C. and Peterson, C. J. Submitted to *Journal of Ecology*, April 2002.

Summary

1 To examine the relative importance of direct and indirect effects of shading on plant performance, we placed *Passiflora biflora* (Passifloraceae) plants in replicated sunny and shaded areas at a premontane field site in southern Costa Rica, and then experimentally permitted or excluded insect herbivores and/or foraging ants attracted to the leaf extrafloral nectar.

2 After 4.5 mo., plant growth (number of leaves, leaf nodes, and shoots) and reproduction (number of flowers and fruits) were significantly lower in the shade, regardless of the presence of herbivores. Leaf toughness was also significantly reduced by shading, but the number of extrafloral nectaries per leaf was unchanged.

3 Exclusion of herbivores with insecticide (vs. water spray control) resulted in substantially improved plant performance, in both sun and shade. Herbivore abundance, however, was unaffected by the plant's light environment. One measure of herbivory (percent empty leaf nodes) indicated that shaded plants were more damaged, but differences in plant growth indicate that overall herbivore impact was greater in the sun.

4 Exclusion of ants using Tanglefoot had no effect on either herbivore abundance or plant performance, indicating that ants were ineffective plant protectors at the study site. Shading had no effect on ant visitation, although percent empty nodes was significantly lower on shaded plants where ants were permitted, suggesting that ant defense was greater in the shade. Surprisingly, fruit production (in sun only) was significantly lower where ants were permitted.

5 Although not experimentally manipulated, we observed a striking negative impact of parasitoid and predaceous wasps on Heliconiinae eggs and caterpillars, but neither egg parasitism nor wasp abundance were significantly altered by the plant's light environment. Because of the impact of wasps on caterpillars, most herbivory on the experimental plants was caused by flea beetles (Alticinae), although leaf miners (Gracillariidae) and whitefly "pupae" (Aleyrodidae) were also frequent.

6 In summary, variation in *P. biflora* performance was little explained by indirect effects of shading involving phytophagous insects and natural enemies, but was well described by the strongly negative direct effects of shading and specialist herbivores.

Key-words: Ant defense, direct vs. indirect effects, extrafloral nectaries, insect herbivory, tropical forest

Introduction

Light is a very heterogeneous resource in forest communities (Canham *et al*. 1990), and its availability is a major limitation to plant growth, survival, and reproduction (Denslow 1980, 1987; Nicotra *et al*. 1999). It has been hypothesized that competition for light has led to specialization by plants for different light environments and that the resulting niche partitioning of the light gradient has facilitated species coexistence (the "gap partitioning hypothesis": Ricklefs 1977; Denslow 1980, 1987; Sipe & Bazzaz 1995; Brokaw & Busing 2000). Numerous recent studies have searched for physiological differences among plant species (e.g., photosynthetic response or relative growth rate), in part to test the gap partitioning hypothesis (e.g., Mulkey *et al*. 1996; Barker *et al*. 1997; Lei & Lechowicz 1998; Agyeman *et al*. 1999; Poorter 1999). These studies clearly show substantial and diverse *direct* effects of light, thereby suggesting possible mechanisms for niche specialization (Brokaw & Busing 2000). However, the more subtle *indirect* effects of light involving both positive and negative interactions with other organisms have received much less attention, yet may be necessary to understand fully the observed field differences in plant performance (Louda *et al*. 1987; Burger & Louda 1994; Louda & Rodman 1996). The potential role of these indirect effects may frequently go unrecognized because their ecological impact is masked by strong direct effects, although in many cases, indirect effects may be ignored simply because it is assumed they are very weak, and therefore unimportant (Yodzis 1988). Because little is known about the frequency, magnitude, or importance of indirect effects in nature (Strauss 1991; Menge 1995), however, it seems premature to make such assumptions.

As they are generally defined (Strauss 1991; Worthen & Moore 1991; Abrams *et al*. 1996), indirect effects occur when one species (the initiator) influences another (the receiver) via a third intermediary species (the transmitter). Thus, if indirect effects are important, variation in some aspect of the initiator species will be useful to explain variation in the receiver species, even if no direct interaction exists between the two. Removal experiments are most often used to infer the interaction strength; for example, by observing the response of the receiver following removal of the transmitter or the initiator, one can evaluate the net magnitudes of both direct and indirect effects. Usually population density is the effect variable of interest, although other kinds of indirect effects also exist (e.g., behavioral effects: Strauss 1991; Abrams *et al*. 1996). In complex systems, the measured net indirect effects are often composites of multiple individual interactions, and the interacting species are typically grouped into "trophospecies" (*sensu* Abrams *et al*. 1996). Therefore, in such situations it is often necessary to monitor a variety of response variables (e.g., abundance and behavior of the interacting organisms) to understand the specific pathways and mechanisms involved (Strauss 1991; Abrams *et al*. 1996; Schmitz *et al*. 2000).

In the study reported here, we experimentally excluded insect herbivores and/or foraging ants from access to *Passiflora biflora*, a Neotropical vine, which we had placed in contrasting light environments (sun vs. shade). We then observed the effects of the shading and exclusion treatments on plant growth and reproduction to determine the impact of various trophospecies interactions (schematically illustrated in Fig. 2.1). Based on the well-known natural history of *Passiflora* (e.g., Gilbert 1991; Benson *et al*. 1975; Benson 1978; Gilbert & Smiley 1978; Smiley 1978,1986; Brown 1981; McLain 1983; Spencer 1988; Apple & Feener 2001), we predicted that the direct effects of shading (Fig. 2.1, arrow #1) and herbivores (arrow #5) would both strongly reduce plant performance and that the direct effect of foraging ants, attracted to the numerous leaf extrafloral nectaries (EFNs), would reduce insect herbivores and herbivory (arrow #7; *sensu* Bentley 1977a).

In addition to these direct effects, the study design also enabled us to determine the existence and magnitude of some indirect effects (see dashed arrows in Fig. 2.1). Specifically, we predicted that if indirect effects of shading are present, the net effect of the plant's light environment would depend on the presence/absence of insect herbivores or ants, and conversely, that the impact of the herbivores and ants would be modified by the plant's light environment. Although light may alter insect behavior directly (e.g., Scriber & Slansky 1981; Mazza *et al*.

1999; arrows #2 and #3), we hypothesized that the primary pathway for light-induced indirect effects (arrows #10-12) would be via changes in the plant's physical, chemical, and biotic defenses, i.e., a "bottom-up" perspective (Hunter & Price 1992). For example, based on the carbon/nutrient balance (CNB) hypothesis (Bryant *et al*. 1983), carbon-based defenses (e.g., leaf toughness and extrafloral nectar) are expected to decline in the shade, whereas nitrogen-based defenses (e.g., leaf cyanogenesis) are expected to increase (but see Hamilton *et al*. 2001). Thus, we expected generalist herbivores to have a greater impact on sun plants, and specialist herbivores of *P. biflora* to have a greater impact on shaded plants, but that the overall impact of herbivores would depend on the relative abundance of generalists and specialists. Moreover, if ants play a role as mediators of an indirect effect of shading, we also predicted that their exclusion from shaded plants would result in less of a negative effect on plant performance than their exclusion from sun plants, possibly because ant defense (arrow #7) is lower in the shade due to reduced extrafloral nectar production (arrow #8).

In this paper, we address the following five major questions about the *P. biflora* tritrophic system (*sensu* Price *et al*. 1980):

1. Does shading have a negative *direct* effect on *P. biflora* growth and reproduction (arrow #1) when the potential indirect effect of herbivores is removed?
2. Do insect herbivores have a negative *direct* effect on plant performance (arrow #5) when the potential defensive role of foraging ants is prevented?
3. Do foraging ants have a negative *direct* effect on herbivore abundance and herbivory (arrow #7) when the potential influence of variation in the light environment is controlled?
4. Does ant access to *P. biflora* have a net positive *indirect* effect on plant performance (arrow #9)?
5. Does shading of *P. biflora* have a net positive or net negative *indirect* effect on plant performance, mediated by an interaction with either herbivores or ants (arrow #12)?

Finally, we compare the amount of variability associated with each interaction (using ANOVA) to examine the relative importance (*sensu* Welden & Slauson 1986) of the direct and indirect effects of shading on *P. biflora*. For improved clarity, we refer throughout the paper to the numbered interactions in Fig. 2.1.

Materials and methods

STUDY SITE AND ORGANISMS

This study was carried out within the grounds of the Robert & Catherine Wilson Botanical Garden in southern Costa Rica, which is a part of the Las Cruces Biological Station (8° 47' N, 82° 57' W; elevation ca. 1100 m). Mean annual rainfall at the site is ca. 4 m, with a variable 3-5 mo. dry season that typically starts in December. The Garden is maintained by periodic mowing and trimming, but no pesticides are used. Most groups of experimental plants (see below) were placed within 10 m of the adjacent secondary premontane rain forest (Hartshorn 1983).

Passiflora biflora Lam. (Passifloraceae) is a common herbaceous vine of old fields and successional forest (Croat 1978). The particular plants used in this study were propagated from cuttings taken from wild *P. biflora* found within ca. 30 km of the Garden. Scattered across the underside of the bilobed leaves are 2-26 yellow, 1-mm diameter extrafloral nectaries, which exude droplets of clear nectar, particularly from young leaves (G.C. Ward, personal observation).

During the course of this research, we documented numerous species of arthropods on the experimental plants (see Appendix A). The most commonly seen herbivores were Alticinae flea beetles (Coleoptera: Chrysomelidae), leaf miners (Lepidoptera: Gracillariidae), whitefly "pupae" (the sap-feeding scale-like sessile instars; Homoptera: Aleyrodidae), and the eggs and caterpillars of Heliconiinae butterflies (Lepidoptera: Nymphalidae). In addition, we also observed ants, web-building spiders, and predatory and parasitic wasps, some of which we confirmed to be natural enemies of the above herbivores (Appendix A). These arthropods were initially distinguished in the field by differences in coloration and morphology (i.e., "morphospecies"), but we later identified voucher specimens using field guides and taxonomic keys (DeVries 1987; Hanson & Gauld 1995), and by comparison to specimens at the *Instituto Nacional de Biodiversidad* (INBio) in Santo Domingo de Heredia, Costa Rica.

EXPERIMENTAL DESIGN AND TREATMENTS

In March 1998, we placed groups of potted *P. biflora* plants in four sunny and four shaded locations in the Garden. Each group consisted of four plants, except for one shaded location that contained only three ($N = 31$). Within each group, plants were positioned 1.5 m apart in a square array. Pots were buried to the rim to allow the roots to grow through the drain holes into the surrounding soil. A bamboo pole was placed in the center of each pot to support the plant stems (using twist-ties), allowing them to grow to ca. 1.25 m in height. In a given location, all plants were propagated from the same source material, but a different clone was used to populate each location.

To test for the effects of insect herbivores, we randomly selected two plants in each group to hand-spray once per week with 0.0025% (w/v) deltamethrin (Decis® 2.5 EC; AgrEvo, Wilmington, Delaware, USA). As a control, the other two plants in each group were hand-sprayed with water only. In either case, the spray was applied until the upper and lower leaf surfaces were slightly wet, and spraying was always carried out at dawn or dusk to minimize application to the diurnal ants and wasps. Deltamethrin is a phosphorous-free pyrethroid insecticide, but it does contain one nitrogen atom. Despite the potential for an unintended fertilizing effect (Brown *et al*. 1987; Crawley 1989), we selected deltamethrin for use in this experiment because of its extremely high potency, which allowed the use of a very dilute concentration.

To test for the effects of foraging ants, we randomly selected one water-sprayed and one insecticide-sprayed plant in each group to receive a Tanglefoot barrier at the plant base (applied over masking tape wrapped around individual stems). Tanglefoot® Pest Barrier (Tanglefoot Company, Grand Rapids, Michigan, USA) is a sticky, non-drying resin used to prevent access to plants by crawling insects. Periodically, the Tanglefoot was refreshed to maintain its effectiveness, and the grass surrounding the plants, if present, was kept trimmed to about 5-cm height to prevent the formation of bridges across the sticky barriers.

Before placement into their assigned locations, the potted plants were grown for several months in a common area (in full sun). Then just before relocation, we trimmed all plants to similar stem length (ca. 0.8 m) and sprayed them once with Decis to remove any resident arthropods. After relocation, we watered the plants every 2-3 days until the dry season ended in mid-April. Every two weeks throughout the study, we fertilized the plants with a solution of NPK 20-20-20 fertilizer plus micronutrients.

The design of this experiment was a split-plot randomized block 2 x 2 factorial. Group location ($n = 8$) was the blocking factor, light environment (sun vs. shade) was the whole-plot factor, and spray type (water-only vs. insecticide) by Tanglefoot treatment (open vs. protected) were the crossed split-plot factors within each location.

PERIODIC CENSUSES AND OTHER MEASUREMENTS

For 4.5 mo. we periodically quantified plant performance and censused the herbivores and natural enemies that were present on the experimental plants. In particular, we counted the number of leaves, actively growing shoots, axillary flower buds, fully developed flowers, fruits, Heliconiinae butterfly eggs, Heliconiinae caterpillars, beetles, leaves occupied by leaf miners, ants, wasps, and spiders. For butterfly eggs, we recorded egg color (yellow vs. black) to monitor the proportion that were parasitized by *Telenomus* sp. wasps (Scelionidae); Heliconiinae eggs turn from yellow to black when parasitized (G.C. Ward, personal observation). We were unable to identify by inspection which eggs were laid by which butterfly species, except for the eggs of *Dione juno* (also Heliconiinae), which are oviposited in large distinctive clusters containing as many as 107 eggs (G.C. Ward, personal observation). However, the counts of *D. juno* eggs were excluded from our analysis because of this distinct oviposition behavior (other Heliconiinae laid eggs singly) and because their egg clusters were only found on the two insecticide-sprayed plants in one sunny location and therefore were quickly killed by the spraying. Also, *D. juno* female butterflies were likely attracted to this one location because of its nearness to a patch of introduced *P. coccinea* (ca. 10 m away) onto which they frequently oviposited. Occasionally individual eggs of other Heliconiinae species were found affixed to the bamboo posts used to support the *P. biflora* plants (or on associated spider webs!); these eggs were included in the counts because they were probably laid in response to the attached plant. Ants were also observed on these support posts, but they were not included in the ant counts because these posts may represent distinct ant foraging areas. To quantify wasp abundance, we approached each plant slowly to avoid disturbing the insects present, first counting the rapidly moving individuals (usually vespids) from ca. 1 m away, and then moving in closer to count the smaller, more sedentary wasps on the undersides of leaves. Each plant was watched for ca. 2 min. during each census. Dipterans were the only major group of arthropods that we did not quantify because their occurrence seemed to have no relationship to *P. biflora* (e.g., we never saw them interacting with other arthropods on the plant), although they may be important prey for the resident spiders.

We were usually unable to quantify plant features and arthropod abundances during the same census because we were simultaneously conducting a companion study of *P. biflora* (Ward & Peterson, unpublished manuscript); thus, census dates were somewhat irregularly distributed throughout the 4.5-mo. study period. Within each census, however, the order of group visitation was always randomized. For the following analyses, we summed the multiple flower, fruit, and arthropod counts across census dates to obtain cumulative totals per plant. Flower buds, fully developed flowers, fruits, ants, and wasps were censused twice; Heliconiinae eggs and caterpillars were counted four times; beetles were counted on five occasions; and spiders were counted only once. For leaves, leaf nodes, and actively growing shoots, we analyzed only the final counts at the end of the study. To determine whether number of EFNs *per leaf* responded to the light environment, we counted EFNs on young fully expanded leaves (N = 187 leaves, 2-12 leaves per plant) on a single occasion midway during the experiment. We then used the per-plant average number of EFNs per leaf in the analysis.

To quantify light availability (i.e., photosynthetic photon flux density, PPFD) in the eight group locations, we used a Sunfleck Ceptometer (model SF-80; Decagon Devices, Inc., Pullman, Washington, USA) held horizontally above each plant, immediately followed by a paired measurement taken at a nearby location in full sun. These measurements were taken in the late morning, during periods of no cloud cover.

LEAF HARVEST

At the end of the study in late July 1998, we harvested all leaves from the experimental plants for final measurements. Leaf area of each leaf was measured using a moving-belt area meter (model LI-3100; Li-Cor, Inc., Lincoln, Nebraska, USA) and herbivore damage was visually estimated using several damage classes (see HERBIVORY MEASUREMENT below). To quantify leaf toughness, we randomly selected three mature leaves per plant and then used a McCormick Fruit Firmness Pressure Tester (the 0-500 g model and 2.5-mm diameter tip; McCormick Fruit Tech, Yakima, Washington, USA) to take 10 readings per leaf, avoiding major veins. (Measured leaves were clamped in a leaf holder made from two sheets of perforated acrylic.) We also randomly selected three other mature leaves per plant to quantify the density of whitefly pupae on the leaf underside. The minute, first-instar larvae were not counted because of their near invisibility to the naked eye.

HERBIVORY MEASUREMENT

We used three methods to quantify herbivory on the experimental plants: (1) In mid-April, we marked a subset of undamaged, young, fully expanded leaves with numbered tags (2-12 leaves per plant, representing 13% to 86% of the total present), and then at leaf harvest, we determined which of these leaves had survived, thereby calculating the *percent survival of marked leaves* per plant. (2) At leaf harvest, we estimated percent herbivory on all extant leaves by visually classifying insect damage into the following six damage classes: 0 - 1%, 1 - 12.5%, 12.5 - 25%, 25 - 50%, 50 - 75%, and 75 - 100%. But because most herbivore damage was minor on the harvested leaves, we pooled these visual estimates into the following two classes: (a) little to no damage (≤ 1% leaf area) and (b) some evident damage (> 1% leaf area). From these counts, we then calculated the *percent of leaves with herbivore damage* per plant. And (3), also at leaf harvest, we counted how many leaf nodes were missing leaves to determine the *percent of empty nodes* per plant. We quantified this variable because early in the study we had noticed that developing (i.e., "premature") leaves tended to abscise when damaged by herbivores, although we acknowledge that leaves may also be shed for reasons other than herbivory (e.g., abiotic causes; Chabot & Hicks 1992).

STATISTICAL ANALYSIS

We used a mixed model ANOVA to assess the impact of treatments on the quantified response variables, setting the blocking factor (i.e., group location) as a random effect. All statistical analyses were done with the GLM procedure in *SPSS for Windows*, release 8.0 (SPSS, Inc., Chicago, Illinois, USA), using the type III sum-of-squares method. Data were transformed as necessary to equalize variance and minimize heteroscedasticity. We inferred the existence of a *direct* effect of shading, herbivores, or ants when the relevant ANOVA main effect (i.e., LIGHT, SPRAY, or TANGLEFOOT) was significant ($P \leq 0.05$) in the absence of a significant interaction term *and* the treatment factor was known to have a direct interaction with the response variable. We inferred the existence of an *indirect* effect in two circumstances: (1) when a significant ANOVA interaction term was found, suggesting nonadditive effects of two trophospecies on a third (Morin *et al*. 1988; Worthen & Moore 1991; Menge 1995), and (2) when a significant

ANOVA main effect was found for a response variable and a trophospecies that we knew were not interacting directly (e.g., since the ants on *P. biflora* do not eat leaves, a significant TANGLEFOOT effect for *number of leaves* would imply that ants had an *indirect* effect on the plants that was mediated by the insect herbivores).

To evaluate the relative importance (*sensu* Welden & Slauson 1986; Morin *et al*. 1988) of the significant direct and indirect effects, we used partial eta-squared values (η_p^2) that were produced as optional output by the SPSS GLM procedure. Partial eta-squared is the proportion of the total sum of squares (SS) used to compute a specific *F*-test, i.e.,

$$\eta_p^2 = \frac{\mathrm{SS_{effect}}}{\mathrm{SS_{effect}} + \mathrm{SS_{error}}} = \frac{\mathrm{d.f._{effect}} \times F_{\mathrm{effect}}}{\left(\mathrm{d.f._{effect}} \times F_{\mathrm{effect}}\right) + \mathrm{d.f._{error}}},$$

where the "effect" and "error" subscripts correspond to the effect of interest and the particular error term used in the test, respectively (Cohen 1973; Olejnik & Algina 2000). These values are analogous to the coefficient of determination (r^2) in regression analysis. They provide a relative index of the amount of variability accounted for by the effect of interest and overcome some of the limitations of other measures of relative importance (Underwood & Petraitis 1993; Dyer & Letourneau 1999; Olejnik & Algina 2000).

Results

EFFECTIVENESS OF EXPERIMENTAL TREATMENTS

The three experimental manipulations used in this study were highly effective at altering their intended target resource or trophospecies (Table 2.1). First, *light availability* in the sunny locations did not differ from nearby areas in full sun, whereas *light availability* in the shaded locations was reduced by 91.3% (± 3.5% SE; Table 2.1a). Second, the insecticide spray significantly reduced the abundance of all herbivore groups compared to the water-only control (Table 2.1b), except for Heliconiinae caterpillars, which were very uncommon due to high egg parasitism and predation. And third, ant access was completely prevented by the Tanglefoot barriers (Table 2.1c).

However, despite the effectiveness of the Tanglefoot barriers, ant abundance on plants where ants were permitted was never high (Table 2.1c; Fig. 2.2j). We also frequently observed parasitic and predaceous wasps visiting EFNs on the experimental plants, and apparently their impact on some insect herbivores was substantial (see results below). Therefore, the TANGLEFOOT treatment was only effective at manipulating one part of the natural enemy trophic level (i.e., ants but not wasps). Also, although the insecticide-spray treatment had no significant effect on the number of wasps (Fig. 2.2k; Table 2.2k) or wasp parasitism of Heliconiinae eggs (data not shown), it did reduce ant (Fig. 2.2j; Table 2.2j) and spider abundance (data not shown). Therefore, besides its effect on herbivory, the insecticide spray treatment may have also reduced the plant's overall level of biotic defense.

DIRECT EFFECTS OF SHADING ON PLANTS (*ARROW #1*)

The direct effects of shading on *P. biflora* performance are revealed by comparing the sun and shaded plants that were protected with insecticide. The isolated effect of shading substantially reduced both plant growth and reproduction: shaded plants had fewer leaves (Fig. 2.2a; Table 2.2a), leaf nodes (i.e., extant leaves + empty nodes; Fig. 2.2c; Table 2.2c), and actively growing shoots (Fig. 2.2d; Table 2.2d). Moreover, shaded plants produced no developed flowers or fruits (Fig. 2.2e; Table 2.2e), although they did produce a few flower buds (data not shown). (Note: the two significant interactions terms in Table 2.2e are artifacts resulting from the complete absence of fruits on shaded plants; Table 2.3 provides a separate analysis of fruit production for the sun plants only.) In contrast to shaded plants, the sun plants produced a number of fully developed flowers (mean ± 1 SE for the insecticide + Tanglefoot plants only [*n* = 4]: 3.8 ± 1.49 per plant) and fruits (11.8 ± 4.71 per plant), as well as numerous flower buds (77.8 ± 34.7 per plant). As indicated by the fewer flowers than fruits, some flowers were apparently not counted because they developed between censuses. Of the measured plant growth responses, the only one that was not significantly reduced by the direct effect of shading was *total leaf area* (P = 0.156; Fig. 2.2b; Table 2.2b), possibly because shaded plants tended to have larger leaves (data not shown).

Besides the above differences in plant growth and reproduction, shading also affected leaf toughness, with leaves on the sun plants being significantly tougher than leaves on the shaded plants (mean ± 1 SE: 572 ± 12 kPa [n = 16] vs. 387 ± 17 kPa [n = 15]; $F_{1,6.017}$ = 26.942, P = 0.002). The average number of EFNs per leaf, however, was only slightly lower on shaded plants (mean in sun vs. mean in shade: 10.2 [n = 16] vs. 9.1 [n = 15]) and not significantly so (P = 0.400). Shading also had no direct effect on rates of leaf abscission, based on our observations of *percent survival of marked leaves* (Fig. 2.2g; Table 2.2g) and *percent of empty nodes* (Fig. 2.2i; Table 2.2i).

DIRECT EFFECTS OF SHADING ON HERBIVORES (*ARROW #2*) AND NATURAL ENEMIES (*ARROW #3*)

The direct effects of shading on herbivores and natural enemies (e.g., avoidance of high temperatures; Scriber & Slansky 1981) were not explicitly tested in this study because we did not control for simultaneous shade-induced changes in the interacting trophic levels (e.g., changes in plant quality or prey abundance). Therefore, our observations on the effect of shading on arthropod abundance represent the potential combined influences of both direct and indirect effects, as is the case for many previous studies of herbivore distribution patterns (e.g., Lincoln & Mooney 1984). Thus, although we present in this section some of our observations on the distribution of arthropods as a direct effect of shading, it is important to recognize that these results may reflect a mixture of both direct and indirect effects of the plant's light environment.

Despite the large differences in light environment and plant characteristics (arrow #1), neither the total number of eggs laid by Heliconiinae butterflies nor the abundance of any herbivore group differed significantly between sun and shaded habitats ($P > 0.05$), although four of the five herbivore groups tended to be less abundant on the shaded plants. Specifically, shaded plants tended to have a lower *cumulative number of Heliconiinae butterfly eggs* (mean in sun vs. mean in shade for the water-spray plants with Tanglefoot [$n = 4$]: 3.25 vs. 0.75 per plant; Fig. 2.2f; Table 2.2f), *cumulative number of Heliconiinae caterpillars* (0.50 vs. 0.25 per plant), *cumulative number of beetles* (4.75 vs. 1.25 per plant), and final *whitefly "pupae" density* (53.94 per dm^2 vs. 0.15 per dm^2). In contrast, shaded plants tended to have a greater *percent of leaves occupied by leaf miners* at harvest (mean in sun vs. mean in shade [$n = 4$]: 1.00% vs. 4.62% per plant), although again this effect was not significant because of high variation among groups.

Similarly, the plant's light environment had little influence on the abundance of putative natural enemies of the insect herbivores ($P > 0.05$). Ants and wasps were only slightly more abundant on the sun plants (Figs 2.2j-k; Tables 2.2j-k), where percent parasitism of Heliconiinae eggs was also somewhat greater (mean in sun vs. mean in shade: 19.81 vs. 8.00 per plant), although spiders tended to be slightly more abundant on shaded plants (0.56 vs. 1.00 per plant).

DIRECT EFFECTS OF PLANTS ON NATURAL ENEMIES (*ARROW #8*)

Although ants were less abundant than expected, we often observed ants and wasps consuming *P. biflora* extrafloral nectar, thus demonstrating the potential for a positive direct interaction between *P. biflora* and these natural enemies. In particular, Vespids were frequently observed traplining for secreted nectar, returning repeatedly to the same set of leaves to collect new secretions.

DIRECT EFFECTS OF NATURAL ENEMIES ON HERBIVORES (*ARROW #7*) AND OTHER NATURAL ENEMIES

We found no direct effect of ant exclusion on herbivore abundance, indicating that the low ant visitation that occurred was ineffective at deterring *P. biflora* herbivores. Specifically, neither Heliconiinae butterfly oviposition (Fig. 2.2f; Table 2.2f) nor the abundance of any herbivore group (data not shown) was significantly affected by the TANGLEFOOT treatment. Most ants counted were small (< 1.5-mm long), and we never observed them showing interest in either butterfly eggs or other arthropods.

In contrast, wasps appeared to have a great impact on Heliconiinae eggs and possibly their caterpillars. Overall, 26.0% of the 77 Heliconiinae eggs counted were parasitized by wasp parasitoids (i.e., *Telenomus* sp.). And because only six Heliconiinae caterpillars were seen throughout this study, it appears that most butterfly eggs were eventually parasitized or that the early instar caterpillars were eaten soon after hatching. On two occasions, we watched small Heliconiinae caterpillars on *P. biflora* being attacked and killed by the same predaceous wasps that trapline for extrafloral nectar (i.e., *Polybia* spp., Vespidae).

Although this interaction is not diagrammed in Fig. 2.1, the Tanglefoot treatment also allowed us to test for an interaction between ants and wasps and between ants and spiders. Yet despite the complete exclusion of ants by the Tanglefoot barriers, we detected no influence of this treatment on *cumulative number of wasps* (Fig. 2.2k; $F_{1,17} = 0.229$, $P = 0.638$, $\eta_p^2 = 0.013$), *percent Heliconiinae egg parasitism* ($F_{1,17} = 0.810$, $P = 0.381$, $\eta_p^2 = 0.045$), or *number of spiders* ($F_{1,17} = 0.016$, $P = 0.900$, $\eta_p^2 = 0.001$), which suggests that intraguild interference did not occur between ants and these other natural enemies, whether by direct or indirect means (e.g., involving food resources).

DIRECT EFFECTS OF HERBIVORES ON PLANTS (*ARROW #5*)

Despite much butterfly oviposition (Fig. 2.2f; Table 2.2f), the vast majority of leaf and shoot damage on the experimental plants was caused by an assortment of flea beetles (Appendix A). Although the insecticide spray treatment had no apparent effect on butterfly oviposition (Fig. 2.2f; Table 2.2f), it did reduce all other herbivore groups. Specifically, compared to the water-only sprayed plants, the insecticide sprayed plants had a lower *cumulative number of beetles* per plant (mean ± 1 SE for Tanglefoot plants only: 3.00 ± 1.10 [n = 8] vs. 0.14 ± 0.14 [n = 7]; $F_{1,17}$ = 18.578, P = 0.0005, η_p^2 = 0.522), lower *percent of leaves occupied by leaf miners* at harvest (2.81 ± 1.41% vs. 0.23 ± 0.23%; $F_{1,17}$ = 5.600, P = 0.030, η_p^2 = 0.248), and a lower *whitefly "pupae" density* at harvest (27.05 ± 24.85 per dm^2 vs. 0.00 ± 0.00 per dm^2; $F_{1,17}$ = 9.400, P = 0.007, η_p^2 = 0.356). Known generalist herbivores (e.g., grasshoppers and slugs) were rarely observed on the experimental plants and caused little plant damage.

Besides its effect on herbivore abundance, the insecticide treatment also strongly reduced insect herbivory. Specifically, plants sprayed with insecticide had a significantly lower *percent of leaves with herbivore damage* (Fig. 2.2h; Table 2.2h) and *percent of empty nodes* at harvest (Fig. 2.2i; Table 2.2i) than did water-only sprayed plants. *Percent survival of marked leaves*, however, was not influenced by the spray treatment (Fig. 2.2g; Table 2.2g), possibly because herbivore-induced leaf abscission occurred mostly to immature leaves, whereas we marked only fully expanded leaves.

In response to the insecticide exclusion of insect herbivores, *P. biflora* produced significantly more leaves (Fig. 2.2a; Table 2.2a), total leaf area (Fig. 2.2b; Table 2.2b), leaf nodes (Fig. 2.2c; Table 2.2c), and actively growing shoots (Fig. 2.2d; Table 2.2d). The insecticide application also significantly increased fruit production, although this effect was only apparent on sun plants because no fruits were produced by shaded plants (Fig. 2.2e). The number of flower buds and fully developed flowers also tended to be greater on insecticide-sprayed plants (data not shown), but here the effect of the SPRAY treatment was not significant (flower buds: $F_{1,17}$ = 2.781, P = 0.114, η_p^2 = 0.141; fully developed flowers: $F_{1,17}$ = 1.572, P = 0.227, η_p^2 = 0.085).

INDIRECT EFFECTS OF NATURAL ENEMIES ON PLANTS (*ARROW #9*)

Ants and other natural enemies of *P. biflora* herbivores may have an indirect effect on plant performance if they serve as plant "bodyguards" (Bentley 1977a). To confirm such a role, the exclusion of ants should result in more herbivory and a corresponding decrease in plant growth and reproduction. Despite the effectiveness of the Tanglefoot barriers, however, ant exclusion had no significant effect on *percent survival of marked leaves* (Fig. 2.2g; Table 2.2g) or *percent of leaves with herbivore damage* (Fig. 2.2h; Table 2.2h), indicating that the ants we observed on *P. biflora* were poor plant protectors or too rare to provide any benefit. But on only shaded plants, *percent of empty nodes* at harvest was significantly higher when ants were excluded (Fig. 2.2i; Tables 2.4a-b), indicating that some plant protection by ants may occur. When only water-sprayed plants are considered, *percent of empty nodes* on shaded plants with and without Tanglefoot differed by 5.6%, whereas the *percent of empty nodes* on sun plants with and without Tanglefoot differed by less than 1%.

However, none of our measurements of plant growth (Figs 2.2a-d; Tables 2.2a-d) were significantly affected by the Tanglefoot treatment, which is consistent with the mostly non-significant effects of Tanglefoot on herbivore abundance and herbivory (see above). In addition, cumulative number of flower buds and developed flowers were also unaffected by ant exclusion (data not shown), but *cumulative number of fruits* was significantly lower on sun plants where ants were permitted (Fig. 2.2e; Table 2.3), indicating that ants may negatively affect plant reproduction—an unexpected result.

INDIRECT EFFECTS OF SHADING ON HERBIVORES (*ARROW #11*) AND NATURAL ENEMIES (*ARROW #10*)

An indirect effect of shading on insect herbivores could occur if the attractiveness of *P. biflora* to herbivores was mediated by shade-induced changes in plant chemistry or ant/wasp visitation. In such a situation, we would expect the abundance and impact of herbivores to depend on whether the host plants were shaded or not. In our study, this kind of indirect effect would be suggested by a significant LIGHT x SPRAY interaction term. However, because we did not control for variation in adjacent trophospecies, a direct effect of shading on insect herbivores (arrow #2) might also explain such a result (see above). Therefore, our observations of herbivore abundance and impact may represent the combined influence of both direct and indirect effects.

Yet given this caveat, the only evidence we found for a possible indirect effect of shading on herbivores (indicated by a significant LIGHT x SPRAY interaction) was for *percent of empty nodes*, which showed that the proportion of leaf abscission depended on the plant's light environment (Fig. 2.2i; Tables 2.4c-d). Although leaf abscission may be caused by biotic and abiotic forces unrelated to herbivory (Chabot & Hicks 1982), it appears that the observed variation in *percent of empty nodes* between sun and shaded plants was related solely to the action of herbivores because *percent of empty nodes* did not differ between sun and shade when the plants were sprayed with insecticide (Fig. 2.2i). By subtracting the average *percent of empty nodes* on insecticide-sprayed plants from the average percentage on water-sprayed plants (considering only the Tanglefoot plants), we estimate that 12.5% of leaves on sun plants were lost to herbivory, whereas 23.5% were lost to herbivory in the shade. However, this result of greater herbivory in the shade contrasts with the pattern of herbivory suggested by average *number of leaves* per plant (Fig. 2.2a), where by subtracting the average *number of leaves* on water-sprayed plants from the average number on insecticide-sprayed plants (again considering only those with Tanglefoot), we estimate that herbivores reduced the number of leaves on the sun plants by 36.1% and the shaded plants by 28.1%. Similar calculations for *total leaf area* (Fig. 2.2b) and *number of actively growing shoots* (Fig. 2.2d) also suggest that herbivores had a greater impact on plants in the sun than in the shade.

As for herbivores, an indirect effect of shading on natural enemies (i.e., ants, wasps, and spiders) could be important if their abundance and impact depended on the plant's light environment, either because of plant-mediated and/or herbivore-mediated interactions. Such an indirect effect involving ants would be suggested in our experiment by a significant LIGHT x TANGLEFOOT interaction term. But again, because we did not control for variation in adjacent trophospecies (specifically herbivores), our observations of abundance and impact of natural enemy may represent the combined influence of both direct and indirect effects of variation in the light environment. Nevertheless, the only indication that natural enemies were influenced indirectly by shading was the presence of a significant LIGHT x TANGLEFOOT interaction for *percent of empty nodes* (Fig. 2.2i; Table 2.2i), which suggests that ants protected shaded plants against herbivore-induced leaf abscission, but not the sun plants (Tables 4a-b).

INDIRECT EFFECTS OF SHADING ON PLANTS (*ARROW #12*)

An overall net indirect effect of shading on *P. biflora* would be indicated by a significant LIGHT x SPRAY or LIGHT x TANGLEFOOT interaction for some measure of plant performance. Despite strong direct effects of shading, however, herbivores, and wasps, the suggested differences in herbivory and ant defense (see above) failed to have a significant effect on any aspect of plant growth or reproduction.

Discussion

In this study, we found strong negative direct effects of shading and insect herbivory on *P. biflora* and a relatively weak indirect effect of shading mediated by ants and insect herbivores. In the following sections, we consider each of the above interactions in turn and then attempt to provide a holistic perspective of the *P. biflora* tritrophic system (*sensu* Price *et al*. 1980).

DIRECT EFFECTS OF SHADING ON PLANTS (*ARROW #1*)

The direct effects of 91% shading on *P. biflora* performance were strongly negative, as expected, resulting in about two-thirds fewer leaves on shaded plants and inhibiting all reproduction. We also observed that shading induced the production of noticeably thinner and softer leaves, which is a well-known plant response to reduced light availability (Corré 1983). Although we were unable to quantify leaf cyanogenesis in this study, we believe it is likely that the large differences in light availability also caused changes in *P. biflora* leaf chemistry. Little is known about the effect of shading on cyanogenesis, but in two unrelated species, *Pteridium aquilinum* (Jones 1983) and *Trifolium repens* (Vickery *et al*. 1987), the degree of cyanogenesis was found to be greater under increased shading. These observations are consistent with the CNB hypothesis (Bryant *et al*. 1983; Gleadow *et al*. 1998), which predicts that allocation to nitrogen-based defense compounds will increase in low-light conditions. Future studies are needed to address the plasticity of *Passiflora* cyanogenesis because these chemical compounds are thought to be of central importance to the interactions with Heliconiinae butterflies and other specialist herbivores (Benson *et al*. 1975; Spencer 1988).

Besides leaf toughness, we also tested the effect of shading on the number of EFNs per leaf. We believe that our study is the first to investigate this potential effect of a plant's light environment. Although we observed much variation in EFN number, ranging from 2-26 EFNs per leaf, we found no evidence that shading explained any of this variation. At the whole-plant level, however, plants in the sun clearly had more EFNs than plants in the shade, simply because sun plants had many more leaves. Shading may also alter nectar secretion by individual nectaries, but this question remains to be studied.

DIRECT EFFECTS OF SHADING ON HERBIVORES (*ARROW #2*) AND NATURAL ENEMIES (*ARROW #3*)

Although the direct effects of shading on herbivores and natural enemies were not explicitly tested in this study, we found no strong indication that the distribution of either herbivores or natural enemies on *P. biflora* was directly altered by the plant's light environment. Flea beetles, Heliconiinae eggs and caterpillars, whitefly pupae, ants, and wasps were all on average slightly less abundant on the shaded plants, but such variation could be due to changes in plant characteristics, not simply direct effects of lower light exposure.

DIRECT EFFECTS OF PLANTS ON NATURAL ENEMIES (*ARROW #8*)

Since Bentley's (1977a) seminal review, it has almost become dogma to assume that EFNs are adaptations to attract ants, which then serve as plant bodyguards against attacking herbivores. This was also our expectation at the beginning of the present study. Although we observed ants on the experimental plants the first day following their re-location to the sunny and shaded areas, the ant abundance averaged only 7.0 per census for sun plants and 2.3 per census for shaded plants (when sprayed with water only and without Tanglefoot). In another study of *P. biflora* in Costa Rica, Apple & Feener (2001) found a much higher frequency of ant visitation (5.7 ants per shoot tip). We believe the lower abundance of ants on *P. biflora* at the Wilson Botanical Garden was a function of the higher elevation of our study site (1100 m); the ant counts by Apple & Feener (2001) were made on plants at the La Selva Biological Station, which is located at ca. 100 m elevation. Several researchers have documented the tendency for ant abundance to decline with increasing elevation and have attributed the distribution pattern of ants on tropical mountains to their low tolerance for colder temperatures (Olson 1994). Bentley (1977b) also noted that ants near the Wilson Botanical Garden were relatively scarce and non-aggressive, and she attributed the loss of EFNs from *Bixa orellana* (Bixaceae) growing in this area to the correlated decline in ant defense.

Koptur (1985) also found a similar elevational pattern in Costa Rica for ants on two species of *Inga* (Fabaceae): at a lowland site (600 m elevation) ants were abundant and effective plant bodyguards, but at an upland location (1300-1650 m) ants were relatively scarce and ineffective bodyguards. She also found that parasitoid abundance and parasitism of the herbivores on *Inga* showed the reverse trend, being greatest on plants at the upland locations (Koptur 1985). Wasp parasitism of Heliconiinae eggs on *P. biflora* may also increase with elevation; for example, neither Smiley (1978) nor Harper (1991) found any parasitism of Heliconiinae eggs on *P. biflora* at the La Selva Biological Station, whereas we frequently observed egg parasitism at the Wilson Botanical Garden. This apparent decline in parasitism rate at lower elevations may be a direct or indirect consequence of the higher ant abundance there and needs to be further investigated. For example, it would be interesting to know whether the overall top-down effect of ant exclusion is partly masked by intraguild interactions between ants and wasps in lowland habitats.

DIRECT EFFECTS OF NATURAL ENEMIES ON HERBIVORES (*ARROW #7*) AND OTHER NATURAL ENEMIES

We found no evidence that the ants on *P. biflora* had any effect on the insect herbivores or other non-ant natural enemies that were also present (i.e., wasps and spiders), except for the apparent reduction in herbivore-caused leaf abscission on shaded plants (see discussion of *percent of empty nodes* below). The lack of an effect on herbivores was unexpected because a protective role of ants on *Passiflora* has been documented previously (McLain 1983; Smiley 1986). Although it is usually assumed that ants are the primary mortality agents of insect herbivores on plants with EFNs, we observed at the Wilson Botanical Garden that most plant protection against Heliconiinae caterpillars was due to wasp parasitoids and predators. Parasitoids and non-ant predators are often observed on plants with EFNs (Hespenheide 1985; Koptur 1985; Pemberton & Lee 1996; Ruhren & Handel 1999), but their protective role has been very little studied and probably underestimated in most systems. These non-ant visitors are typically much more difficult to manipulate experimentally than ants, but our understanding of the functioning of EFNs will remain incomplete until their role as plant defenders has been given more attention. For example, we suggest that plants with EFNs that appear to gain no benefit from ant defense (e.g., O'Dowd & Catchpole 1983; Rashbrook *et al*. 1992; Zachariades & Midgley 1999) may be protected in many cases by non-ant bodyguards that are not excluded by experimental sticky barriers.

DIRECT EFFECTS OF HERBIVORES ON PLANTS (*ARROW #5*)

By excluding insects with insecticide from half of our experimental plants, we demonstrated the strong impact of insect herbivores on *P. biflora*. We believe our study is the first to quantify the magnitude of herbivore impact on a *Passiflora* species in nature, despite the long-standing interest in the *Passiflora*-herbivore system. We also observed in this study that the vast majority of plant damage to *P. biflora* was caused by flea beetles, not by Heliconiinae caterpillars, even though most prior ecological and evolutionary research on *Passiflora* has emphasized the importance of the Heliconiinae interaction. Therefore, we suggest that the role of non-Heliconiinae herbivores in the *Passiflora* "arms race" has been underappreciated.

Two of our measures of herbivory (i.e., *percent of leaves with herbivore damage* and *percent of empty nodes*) were useful at detecting differences in herbivore damage between water-sprayed and insecticide-sprayed plants, but tracking of marked leaves was unable to detect such differences. The latter may have failed because too few leaves were marked, or possibly, because the selected leaves were already fully developed when marked and had therefore escaped the earlier stage of greatest herbivory risk.

Although the importance of herbivory in the tropics is now generally recognized (Coley & Barone 1996), relatively few studies have used experimental methods of herbivore exclusion to investigate the magnitude of herbivore impact on tropical plants. Our study provides such experimental evidence.

INDIRECT EFFECTS OF NATURAL ENEMIES ON PLANTS (*ARROW #9*)

The significant decrease in *percent of empty nodes* on the shaded plants with ant access was the only evidence we found that ants protect *P. biflora* against attacking herbivores.

Surprisingly, this apparent protection occurred despite the tendency for ants to be less abundant on shaded plants, which suggests that their per capita effect in the shade was actually greater than in the sun—possibly because nectar resources are of greater value to ants in these habitats or because different ants or herbivores were present. Because ant access to shaded plants had no detectable effect on any measure of plant growth, however, it appears that although the ants provided some protection, the plants did not benefit (*sensu* de la Fuente & Marquis 1999), apparently because *P. biflora* compensated for the herbivore-caused leaf abscission. Given their rarity, perhaps ants on *P. biflora* in upland locations are largely parasites of an underappreciated plant-wasp mutualism (see discussion above). We did not experimentally manipulate wasp access, but our observations of wasp impact on Heliconiinae suggest that *P. biflora* performance at the Wilson Botanical Garden is improved by the presence of these natural enemies. By attacking early life history stages, wasp egg parasitoids and larval predators were very effective *P. biflora* bodyguards, unlike the pupal parasitoids (*Conura* spp., Chalcididae; Appendix A), whose presence may not benefit the individual host plant (Koptur 1985). Nevertheless, further investigation is needed to determine the actual magnitude of the top-down impact of wasps. Contrary to the suggestion of several researchers (e.g., Strong 1992; Schmitz *et al*. 2000), we found no evidence that the indirect effect of natural enemies was in any way weakened (i.e., attenuated) by the plant's antiherbivore defenses, given the strong impact of insect herbivores on *P. biflora*.

We detected no beneficial function of ants on *P. biflora*, but we did find evidence that fruit production was somehow reduced by their presence. This surprising negative effect was only apparent on sun plants, as no flowers or fruits were produced in the shade. We occasionally observed flea beetles feeding on *P. biflora* flowers, but it is unlikely that the Tanglefoot barriers excluded these herbivores to give the false impression that ants were responsible for the decreased fruit production; on average, beetles were more abundant on sun plants with sticky barriers. Without further study, however, it is impossible to identify the mechanism behind this apparent negative effect of ants or whether it was caused by a direct interaction with *P. biflora*, i.e., by directly damaging *P. biflora* flowers, or indirectly by deterring pollinator visitation or increasing the plant's allocation to extrafloral nectar. Few researchers have considered the potential for predaceous and scavenging ants to have negative effects on plants with EFNs because it is usually assumed that the nectar secretions "pay" for the cost of the presumed mutualism. In this study, we detected the apparent detrimental effect of ants on fruit production only because it was not masked by a net beneficial role of ant visitation. Thus, in situations with confirmed ant bodyguards, it will be necessary to conduct complex exclusion experiments to isolate the potential negative effects of ant attendance (e.g., Altshuler 1999). Moreover, it should be recognized that ants and wasps may place opposing directional selection pressures, as appears to be the case for *P. biflora* in our study, such that the net direction of selection in a particular population may depend upon the relative abundance of these two natural enemies (Thompson 1994). In such circumstances, we might expect the evolution of traits that deter ants while maintaining wasp visitation. For example, ants are known to have preferences for certain amino acid and sugar concentrations (Lanza 1988); thus, nectar compositions that attract only wasps may provide a mechanism to escape such fitness trade-offs.

INDIRECT EFFECTS OF SHADING ON HERBIVORES (*ARROW #11*) AND NATURAL ENEMIES (*ARROW #10*)

Variation in herbivore abundance or herbivory is commonly found across light availability gradients. Sometimes numbers of herbivores or their damage is greater on plants in sunnier environments (e.g., Lincoln & Mooney 1984; Collinge & Louda 1988; Denslow *et al*. 1990; Louda & Rodman 1996), sometimes greater in shaded environments (e.g., Denslow *et al*. 1990; Burger & Louda 1994; Dudt & Shure 1994), and sometimes no difference is found (e.g., Aide & Zimmerman 1990; Denslow *et al*. 1990; Dudt & Shure 1994; Angulo-Sandoval & Aide 2000). Therefore, it appears unlikely that simple generalizations can be made about the effect of light availability on herbivory (Louda *et al*. 1987). But when variation in herbivory is observed, it is often correlated with light-induced changes in leaf characteristics, including changes in leaf toughness (e.g., Coley 1983; Dudt & Shure 1994), defensive chemistry (e.g., Bryant *et al*. 1983), and nutritional quality (e.g., Scriber & Slansky 1981).

Yet despite the large differences in *P. biflora* growth, leaf toughness, and possibly leaf chemical defenses, the only significant indication of a possible indirect effect of shading on the insect herbivores in our study was the observed increase in *percent of empty nodes* on shaded plants. Based on this result, it is tempting to conclude that overall herbivore impact was less for the sun plants, even though most herbivore groups tended to be more abundant on these plants (except possibly leaf miners). We suspect, however, that this conclusion is incorrect. When the performance of water-sprayed plants is compared with that of insecticide-sprayed plants (for final *number of leaves*, *total leaf area*, and *number of actively growing shoots*), the actual impact of herbivores appears to have been greater in the sun (e.g., the impact of herbivores in the sun resulted in 36.1% fewer leaves vs. 28.1% fewer in the shade). We believe these contrasting results occurred simply because the amount of tissue damage does not necessarily predict actual plant impact, although this relationship is frequently assumed. Thus, in our study, even though sun plants had a lower *percent of empty nodes* than shaded plants, we believe these plants experienced a greater opportunity cost by the loss of individual leaves; in other words, the leaves of sun plants were more profitable than those of shaded plants, and their loss therefore had a greater impact. We predict that eventually this apparent difference in opportunity cost would have resulted in a significant performance difference between water-sprayed and insecticide-sprayed plants, such that if the study had run longer, we would have detected a significant LIGHT x SPRAY interaction for these response variables.

But why didn't shading have a greater and more immediate impact on herbivore abundance and herbivory? Possibly the insect herbivores that were present on *P. biflora* were relatively insensitive to the light-induced changes in plant quality because they were nearly all

Passiflora specialists and therefore well adapted to the physical and chemical variation in their host plants. In habitats dominated by generalist herbivores, light-induced gradients in herbivory may be more common.

Variation in predation and parasitism may also explain patterns of herbivore damage across environmental gradients or, if intense enough, even obscure the effects of variation in host plant quality (Karban 1989). Oksanen *et al*. (1981) predicted that the abundance of natural enemies at the top trophic level would increase with greater ecosystem productivity, resulting in a greater impact of top-down forces on lower trophic levels. In our study, productivity of individual plants was altered by placing *P. biflora* in sunny and shaded environments. We hypothesized that the availability of extrafloral nectar would decline with shading because, as a mostly carbon-based product (Bentley 1977a), extrafloral nectar should be relatively more expensive for shaded plants to produce. Therefore, we predicted that shading would indirectly decrease the abundance and impact of natural enemies, consistent with the ecosystem-level prediction of Oksanen *et al*. (1981). But despite the increase in *number of leaves* on sun plants, and therefore probably overall nectar availability, we found only weak evidence for a corresponding bottom-up increase in natural enemy abundance (i.e., slightly more ants and wasps on sun plants), and based on the results for *percent of empty nodes*, greater light availability appears to have actually decreased the defensive top-down role of ants. We suggest two hypotheses for why our observations of *P. biflora* failed to support the ecosystem-level predictions of Oksanen *et al*. (1981). First, wasp parasitoids and predators may "spill over" from productive to unproductive habitats (Oksanen *et al*. 1992) because their home ranges encompass many plants with EFNs, across a wide variety of light environments. And second, the indirect effect of wasps on *P. biflora* may be attenuated, not by plant antiherbivore defenses (Strong 1992; Schmitz *et al*. 2000), but by the antipredator defenses of flea beetles (e.g., protective elytra, jumping behavior, and cyanogenesis; G.C. Ward, personal observations). Additional studies are needed to investigate the environmental "conditionality" of biotic defense (Bronstein 1994) as mediated by changes in extrafloral nectar production, as well as the ecological relevance of such conditionality.

INDIRECT EFFECTS OF SHADING ON PLANTS (*ARROW #12*)

Overall, we found two lines of evidence that suggest *P. biflora* performance may be indirectly affected by shading (i.e., evidence that the effect of the light environment depended on the presence/absence of insect herbivores or natural enemies). First, realized herbivore impact appeared to be greater on sun plants than on the shaded plants (see discussion above), indicating that herbivores and/or natural enemies were responding to the plant's light environment. In general, indirect effects are expected to take longer to become apparent than direct effects (Yodzis 1988; Menge 1995), so perhaps our experiment did not run long enough for the full impact of herbivores or natural enemies to be manifested. Second, given the strong top-down impact of wasps on Heliconiinae eggs and caterpillars, and the tremendous potential for caterpillars to defoliate plants, the slight oviposition preference of Heliconiinae butterflies for sun plants (Fig. 2.2f) may result in substantially greater herbivore impact on these plants if wasp attack was somehow prevented. Consequently, in the absence of wasps, we predict *P. biflora* performance would be substantially reduced in sunny habitats by the action of these caterpillars.

We further suggest that the net indirect effect of shading on *P. biflora* performance may have been dampened in this study by a spillover effect resulting from high landscape-level heterogeneity in light availability (Oksanen *et al*. 1992; Strong 1992). In these habitats, vagile organisms, such as flying herbivores and wasps, may be little influenced by light-induced differentiation in plant characteristics, making light-induced gradients in herbivory unlikely. This spillover effect may be one of the ecological consequences of fine-scale patchiness in light availability found in many tropical forests (Denslow 1987; Canham *et al*. 1990; Nicotra *et al*. 1999).

RELATIVE IMPORTANCE OF DIRECT AND INDIRECT EFFECTS

We calculated partial eta-squared values (η_p^2) to allow comparison of the magnitude of each direct and indirect effect in this study. Fig. 2.3 shows a summary diagram based on some of these values (*sensu* Dyer & Letourneau 1999) that represents our present working model for the *P. biflora* tritrophic system at the Wilson Botanical Garden. Clearly, plant performance was dominated by a strongly negative bottom-up direct effect of shading (arrow #1), but the top-down direct effect of insect herbivores (arrow #5) also explained much of the variation among plants. In comparison, ants had a negligible effect on herbivore abundance (arrow #7) or plant performance (arrow #9), and the overall indirect effect of shading (arrow #12), as revealed by our herbivore and ant manipulations, was also very weak. Although not explicitly tested in this study, we infer that wasps had a strong negative direct effect on herbivores (i.e., Heliconiinae), but that the magnitude of their effect was probably less than the impact of the insecticide spray treatment, which protected against all herbivore groups. And by extrapolation, we predict that the positive top-down indirect effect of wasps on *P. biflora* is also substantial.

In the last 20 years, the emphasis of ecological research on indirect effects has switched from documenting their existence to determining their relative importance compared to direct effects (see reviews by Schoener 1993; Menge 1995; Abrams *et al*. 1996). After reviewing studies carried out in lakes, rivers, and terrestrial habitats, Schoener (1993) suggested that indirect effects are generally weaker than direct effects, whereas in marine intertidal systems, Menge (1995) concluded that indirect effects explained about as much variation as direct effects. Our results are more in agreement with Schoener (1993) because *P. biflora* performance at the Wilson Botanical Garden was largely determined by the direct effects of shading, insect herbivores, and wasps, and little influenced by the net indirect effects involving herbivores and natural enemies.

Conclusions

Our primary objective was to examine the relative importance of direct and indirect effects of shading on *P. biflora*. Our results demonstrate that *P. biflora* performance in sun and shade is well described by the direct effects of light availability and that the impacts of herbivores and natural enemies, although also very important, appear to be largely independent of the plant's light environment, at least for the short term. Additional research is needed to test the generality of our results for *P. biflora* in other locations and for longer periods, particularly to investigate the influence of elevational variation on the tritrophic interactions. As we have shown here, our understanding of plants with EFNs can be greatly improved by field experiments that simultaneously manipulate both herbivores and natural enemies across an environmental gradient. For this reason, we suggest that it may be more profitable for investigators not to ask "Do the direct effects of light availability on plant performance predict community composition?" (i.e., the "narrow version" of the gap partitioning hypothesis; Sipe & Bazzaz 1995), but rather to ask "How important are the direct effects relative to the indirect effects, and what circumstances alter their relative importance?" (Welden & Slauson 1986; Hunter & Price 1992). The latter question assumes the existence of multiple interacting causes, not the preeminence of direct effects as an explanation for community structure, and it emphasizes the determination of relative importance, not simply intensity, which may be unrelated.

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Table 2.1 Selected responses to the three experimental treatments [mean ± 1 SE (n)] to demonstrate treatment effectiveness. As expected, the experimental manipulations caused significant reductions in the target response variables ($P \leq 0.05$, using mixed model ANOVA), except for Heliconiinae caterpillars, which were very uncommon on the experimental plants. Data transformations used before analysis are indicated in Table 2.2.

a.	**LIGHT treatment**	**Sun**	**Shade**	***P***
	Light availability (PPFD, µmol m^{-2} s^{-1})	2468 ± 86 (16)	226 ± 92 (15)	0.0001
b.	**SPRAY treatment**	**Water-only**	**Insecticide**	***P***
	Cumulative number of Heliconiinae caterpillars	0.31 ± 0.120 (16)	0.07 ± 0.067 (15)	NS
	Cumulative number of beetles	2.25 ± 0.649 (16)	0.20 ± 0.107 (15)	0.001
	Percent of leaves occupied by leaf miners	4.08 ± 2.102 (16)	0.11 ± 0.108 (15)	0.017
	Whitefly "pupae" density (no. per dm^2)	20.52 ± 13.061 (16)	0.00 ± 0.000 (15)	0.007
c.	**TANGLEFOOT treatment**[a]	**Without Tanglefoot**	**With Tanglefoot**	***P***
	Cumulative number of ants	9.25 ± 3.904 (8)	0.00 ± 0.000 (8)	0.023

[a] Based on the water-sprayed plants only.

Table 2.2 ANOVA results for the plant and arthropod responses shown in Fig. 2.2. Each response variable was analyzed using randomized block split-plot 2 x 2 factorial ANOVA; significant sources of variation ($P \leq 0.05$) are in bold. LIGHT (sun vs. shade) is the whole-plot treatment, and BLOCK is the group location (a random effect) nested within LIGHT. SPRAY (water-only vs. insecticide) and TANGLEFOOT (open vs. protected) are the crossed within-plot factors. MS_{error} values are not shown, but can be calculated from the other information. The relative importance of each source of variation is indicated by the partial eta-squared values (η_p^2). Data transformations used before analysis are indicated in the footnotes.

	SOURCE OF VARIATION	d.f.[a]	MS	*F*	*P*	η_p^2
a.	*Number of leaves at harvest*[b]					
	LIGHT	**1, 6.042**	**134.493**	**14.417**	**0.009**	**0.705**
	BLOCK(LIGHT)	**6, 17**	**9.403**	**3.291**	**0.025**	**0.537**
	SPRAY	**1, 17**	**38.567**	**13.496**	**0.002**	**0.443**
	TANGLEFOOT	1, 17	0.325	0.114	0.740	0.007
	LIGHT x SPRAY	1, 17	0.482	0.169	0.686	0.010
	LIGHT x TANGLEFOOT	1, 17	1.187	0.415	0.528	0.024
	SPRAY x TANGLEFOOT	1, 17	1.799	0.630	0.438	0.036
	LIGHT x SPRAY x TANGLEFOOT	1, 17	3.716	1.300	0.270	0.071
b.	*Total leaf area at harvest*[c]					
	LIGHT	1, 6.014	1.076	2.628	0.156	0.304
	BLOCK(LIGHT)	**6, 17**	**0.414**	**10.223**	**0.0001**	**0.783**
	SPRAY	**1, 17**	**0.601**	**14.850**	**0.001**	**0.466**
	TANGLEFOOT	1, 17	0.027	0.671	0.424	0.038
	LIGHT x SPRAY	1, 17	0.056	1.387	0.255	0.075
	LIGHT x TANGLEFOOT	1, 17	0.047	1.173	0.294	0.065
	SPRAY x TANGLEFOOT	1, 17	0.056	1.392	0.254	0.076
	LIGHT x SPRAY x TANGLEFOOT	1, 17	0.055	1.351	0.261	0.074
c.	*Number of leaf nodes at harvest*[b]					
	LIGHT	**1, 6.043**	**105.168**	**9.228**	**0.023**	**0.604**
	BLOCK(LIGHT)	**6, 17**	**11.488**	**3.262**	**0.025**	**0.535**
	SPRAY	**1, 17**	**18.123**	**5.146**	**0.037**	**0.232**
	TANGLEFOOT	1, 17	3.200	0.909	0.354	0.051
	LIGHT x SPRAY	1, 17	0.027	0.008	0.931	0.000
	LIGHT x TANGLEFOOT	1, 17	0.197	0.056	0.816	0.003
	SPRAY x TANGLEFOOT	1, 17	2.190	0.622	0.441	0.035
	LIGHT x SPRAY x TANGLEFOOT	1, 17	2.168	0.615	0.444	0.035

Table 2.2 *continued*

	SOURCE OF VARIATION	d.f.[a]	MS	*F*	*P*	η_p^2
d.	*Number of actively growing shoots at harvest*[d]					
	LIGHT	**1, 6.173**	**1.471**	**6.630**	**0.041**	**0.518**
	BLOCK(LIGHT)	6, 17	0.221	0.808	0.577	0.222
	SPRAY	**1, 17**	**2.188**	**7.992**	**0.012**	**0.320**
	TANGLEFOOT	1, 17	0.000	0.000	0.997	0.000
	LIGHT x SPRAY	1, 17	0.000	0.001	0.971	0.000
	LIGHT x TANGLEFOOT	1, 17	0.207	0.754	0.397	0.042
	SPRAY x TANGLEFOOT	1, 17	0.000	0.001	0.976	0.000
	LIGHT x SPRAY x TANGLEFOOT	1, 17	0.111	0.405	0.533	0.023
e.	*Cumulative number of fruits*[d]					
	LIGHT	**1, 6.019**	**14.168**	**7.233**	**0.036**	**0.546**
	BLOCK(LIGHT)	**6, 17**	**1.978**	**7.403**	**0.001**	**0.723**
	SPRAY	**1, 17**	**3.860**	**14.444**	**0.001**	**0.459**
	TANGLEFOOT	**1, 17**	**1.219**	**4.562**	**0.048**	**0.212**
	LIGHT x SPRAY	**1, 17**	**3.860**	**14.444**	**0.001**	**0.459**
	LIGHT x TANGLEFOOT	**1, 17**	**1.219**	**4.562**	**0.048**	**0.212**
	SPRAY x TANGLEFOOT	1, 17	0.009	0.033	0.858	0.002
	LIGHT x SPRAY x TANGLEFOOT	1, 17	0.009	0.033	0.858	0.002
f.	*Cumulative number of Heliconiinae eggs*[d]					
	LIGHT	1, 6.016	6.235	3.650	0.104	0.378
	BLOCK(LIGHT)	**6, 17**	**1.725**	**8.858**	**0.0002**	**0.758**
	SPRAY	1, 17	0.003	0.002	0.968	0.000
	TANGLEFOOT	1, 17	0.074	0.380	0.546	0.022
	LIGHT x SPRAY	1, 17	0.143	0.736	0.403	0.041
	LIGHT x TANGLEFOOT	1, 17	0.106	0.542	0.472	0.031
	SPRAY x TANGLEFOOT	1, 17	0.636	3.266	0.088	0.161
	LIGHT x SPRAY x TANGLEFOOT	1, 17	0.029	0.151	0.703	0.009
g.	*Percent survival of marked leaves at harvest*[e]					
	No sources of variation with $P < 0.11$					
h.	*Percent of leaves with herbivore damage at harvest*[e]					
	LIGHT	1, 6.064	0.234	3.477	0.111	0.364
	BLOCK(LIGHT)	6, 17	0.068	2.167	0.098	0.433
	SPRAY	**1, 17**	**0.527**	**16.898**	**0.001**	**0.498**
	TANGLEFOOT	1, 17	0.030	0.955	0.342	0.053
	LIGHT x SPRAY	1, 17	0.037	1.196	0.289	0.066
	LIGHT x TANGLEFOOT	1, 17	0.036	1.143	0.300	0.063
	SPRAY x TANGLEFOOT	1, 17	0.013	0.418	0.526	0.024
	LIGHT x SPRAY x TANGLEFOOT	1, 17	0.031	0.042	0.841	0.002

Table 2.2 *continued*

	SOURCE OF VARIATION	d.f.[a]	MS	*F*	*P*	η_p^2
i.	*Percent of empty nodes at harvest*[e]					
	LIGHT	1, 6.008	0.163	1.108	0.333	0.156
	BLOCK(LIGHT)	**6, 17**	**0.148**	**17.269**	**<0.0001**	**0.859**
	SPRAY	**1, 17**	**0.267**	**31.089**	**<0.0001**	**0.646**
	TANGLEFOOT	1, 17	0.024	2.745	0.116	0.139
	LIGHT x SPRAY	1, 17	0.031	3.562	0.076	0.173
	LIGHT x TANGLEFOOT	**1, 17**	**0.079**	**9.187**	**0.008**	**0.351**
	SPRAY x TANGLEFOOT	1, 17	0.008	0.886	0.360	0.050
	LIGHT x SPRAY x TANGLEFOOT	1, 17	0.035	4.083	0.059	0.194
j.	*Cumulative number of ants*[d]					
	LIGHT	1, 6.182	0.835	1.054	0.343	0.146
	BLOCK(LIGHT)	6, 17	0.790	0.767	0.606	0.213
	SPRAY	**1, 17**	**4.801**	**4.660**	**0.045**	**0.215**
	TANGLEFOOT	**1, 17**	**9.792**	**9.504**	**0.007**	**0.359**
	LIGHT x SPRAY	1, 17	1.914	1.857	0.191	0.098
	LIGHT x TANGLEFOOT	1, 17	0.642	0.624	0.441	0.035
	SPRAY x TANGLEFOOT	1, 17	4.321	4.194	0.056	0.198
	LIGHT x SPRAY x TANGLEFOOT	1, 17	2.237	2.172	0.159	0.113
k.	*Cumulative number of wasps*[d]					
	No sources of variation with $P < 0.25$					

[a] Degrees of freedom (d.f.$_{\text{factor}}$, d.f.$_{\text{error}}$). The d.f.$_{\text{error}}$ is adjusted by the ANOVA software to account for the unbalanced design.

[b] $x' = \sqrt{(x)}$. [c] $x' = \log_{10}(x)$. [d] $x' = \sqrt{(x + 0.5)}$. [e] $x' = \text{arcsine}(\sqrt{(x / 100)})$.

Table 2.3 ANOVA results for *cumulative number of fruits* on sun plants only. (No fruits were produced by the shaded plants.) Significant sources of variation ($P \leq 0.05$) are in bold. Data were square-root transformed before analysis.

SOURCE OF VARIATION	d.f.	MS	*F*	*P*	η_p^2
BLOCK	**3**	**3.957**	**7.838**	**0.007**	**0.723**
SPRAY	**1**	**8.149**	**16.144**	**0.003**	**0.642**
TANGLEFOOT	**1**	**2.573**	**5.098**	**0.050**	**0.362**
SPRAY x TANGLEFOOT	1	0.019	0.037	0.852	0.004
Error	9	0.505			

Table 2.4 Reduced model ANOVA results for *percent of empty nodes at harvest*, representing (a) only sun plants, (b) only shaded plants, (c) only plants without Tanglefoot, and (d) only plants with Tanglefoot. Significant sources of variation ($P \leq 0.05$) are in bold. Data were arcsine square root transformed before analysis. Comparison of (a) and (b) indicates that ants protected against leaf abscission in the shade only. Comparison of (c) and (d) indicates that the indirect effect of shading on leaf abscission, revealed by the significant LIGHT x SPRAY interaction, was only apparent on plants where ants were permitted.

	SOURCE OF VARIATION	d.f.	MS	*F*	*P*	η_p^2
a.	*Sun plants*					
	BLOCK	**3**	**0.063**	**8.406**	**0.006**	**0.737**
	SPRAY	**1**	**0.062**	**8.292**	**0.018**	**0.480**
	TANGLEFOOT	1	0.009	1.151	0.311	0.113
	SPRAY x TANGLEFOOT	1	0.005	0.710	0.421	0.073
	Error	9	0.007			
b.	*Shade plants*					
	BLOCK	**3**	**0.234**	**23.687**	**0.0002**	**0.899**
	SPRAY	**1**	**0.227**	**22.989**	**0.001**	**0.742**
	TANGLEFOOT	**1**	**0.090**	**9.070**	**0.017**	**0.531**
	SPRAY x TANGLEFOOT	1	0.036	3.621	0.094	0.312
	Error	8	0.010			
c.	*Plants without Tanglefoot*[a]					
	LIGHT	1, 6[b]	0.008	0.085	0.780	0.014
	BLOCK(LIGHT)	**6, 6**	**0.093**	**10.538**	**0.006**	**0.913**
	SPRAY	**1, 6**	**0.193**	**21.841**	**0.003**	**0.784**
	LIGHT x SPRAY	**1, 6**	**0.069**	**7.853**	**0.031**	**0.567**
d.	*Plants with Tanglefoot*[a]					
	LIGHT	1, 6.051[b]	0.200	3.149	0.126	0.342
	BLOCK(LIGHT)	**6, 5**	**0.068**	**16.248**	**0.004**	**0.951**
	SPRAY	**1, 5**	**0.091**	**22.009**	**0.005**	**0.815**
	LIGHT x SPRAY	1, 5	0.000	0.006	0.939	0.001

[a] MS_{error} values are not shown, but can be calculated from the other information.

[b] $d.f._{factor}$, $d.f._{error}$.

Fig. 2.1 A schematic diagram of the hypothesized direct and net indirect effects of the light environment (i.e., shading) on *Passiflora biflora* and its associated arthropods. The solid lines indicate potential direct effects, and the dashed lines indicate potential net indirect effects. Arrows point from the initiator to the receiver of the interaction, and the sign of the arrow (+, -, or ?) indicates the predicted net outcome to the receiver.

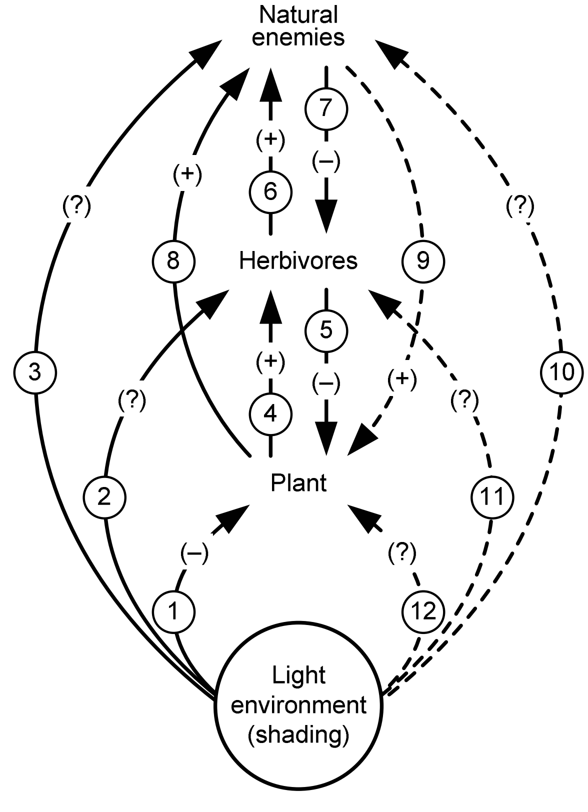

Natural enemies
Herbivores
Plant
Light environment (shading)
1
2
3
4
5
6
7
8
9
10
11
12
(–)
(?)
(?)
(+)
(–)
(+)
(–)
(+)
(+)
(?)
(?)
(?)

Fig. 2.2 Plant and arthropod responses to the experimental treatments (mean ± 1 SE). Each bar is based on $n = 4$ (except $n = 3$ for the rightmost bar). Open vs. filled is used to indicate the application of insecticide spray, whereas open vs. hatching is used to indicate the presence of Tanglefoot barriers. Significant differences among factors are indicated by asterisks followed by the factor name: * $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$. Significant BLOCK effects are not shown (see Table 2.2). Footnotes on significant factors indicate that the statistical result was based on a reduced experimental design due to a significant interaction effect: (1) analysis of the LIGHT effect only using randomized block ANOVA, (2) analysis of the sun plants only using randomized block 2 x 2 factorial ANOVA, and (3) analysis of the shaded plants only using randomized block 2 x 2 factorial ANOVA.

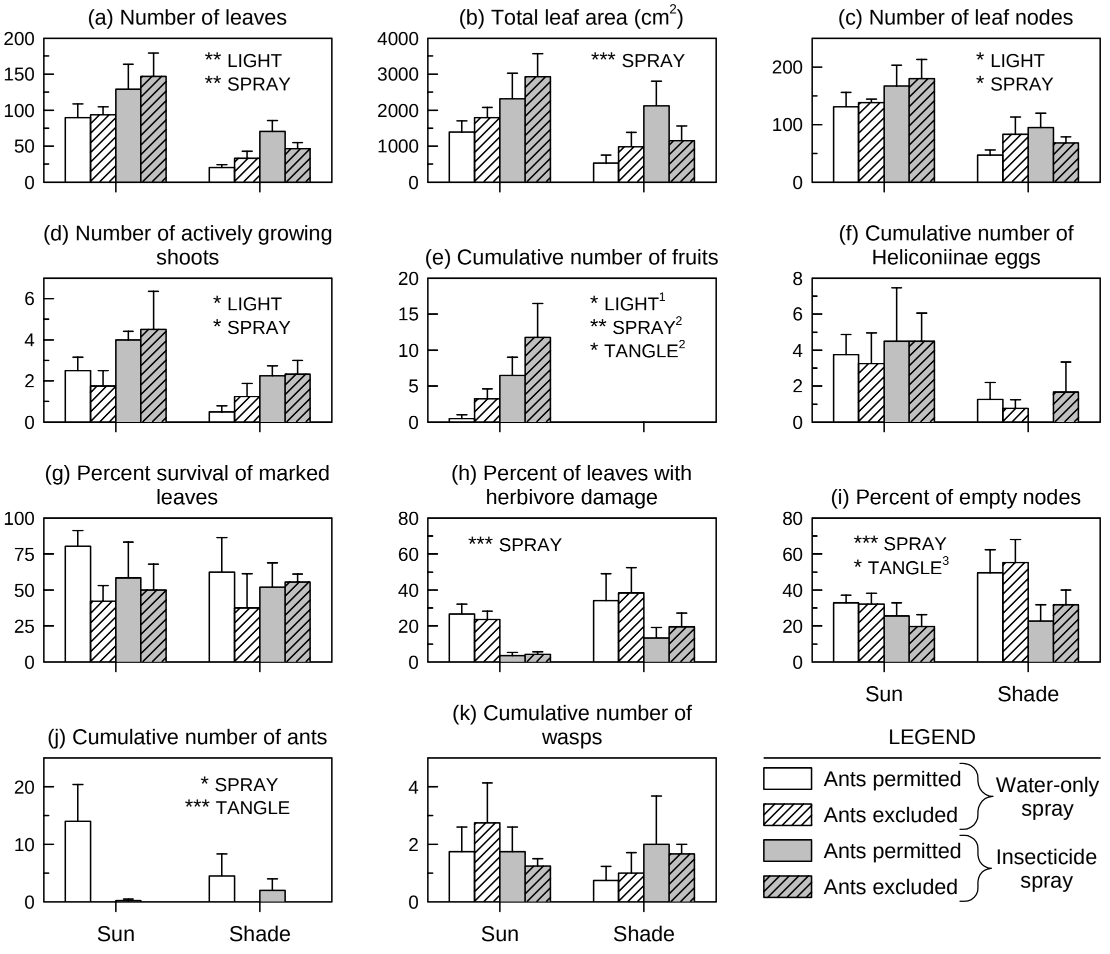
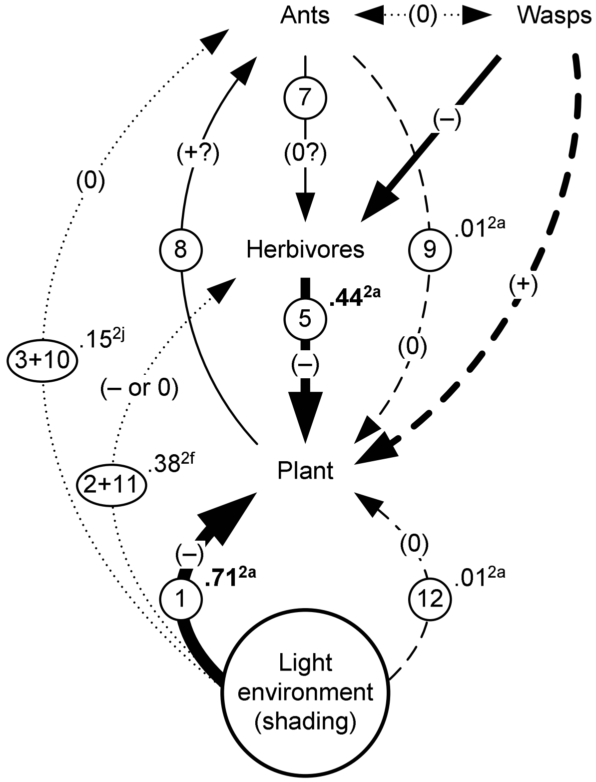

(a) Number of leaves
** LIGHT
** SPRAY
0
50
100
150
200
(b) Total leaf area (cm^2)
*** SPRAY
0
1000
2000
3000
4000
(c) Number of leaf nodes
* LIGHT
* SPRAY
0
100
200
(d) Number of actively growing shoots
* LIGHT
* SPRAY
0
2
4
6
(e) Cumulative number of fruits
* LIGHT[1]
** SPRAY[2]
* TANGLE[2]
0
5
10
15
20
(f) Cumulative number of Heliconiinae eggs
0
2
4
6
8
(g) Percent survival of marked leaves
0
25
50
75
100
(h) Percent of leaves with herbivore damage
*** SPRAY
0
20
40
60
80
(i) Percent of empty nodes
*** SPRAY
* TANGLE[3]
0
20
40
60
80
Sun
Shade
(j) Cumulative number of ants
* SPRAY
*** TANGLE
0
10
20
Sun
Shade
(k) Cumulative number of wasps
0
2
4
Sun
Shade
LEGEND
Ants permitted
Ants excluded
Water-only spray
Ants permitted
Ants excluded
Insecticide spray

Fig. 2.3 Summary diagram of the *P. biflora* tritrophic system, using the calculated partial eta-squared values (η_p^2) to indicate the relative strength of the hypothesized interactions. Solid lines indicate direct effects, dashed lines indicate net indirect effects, and dotted lines indicate combined direct and indirect effects that were not distinguished in this study. Line thickness indicates the strength of the significant interactions, and the sign of the arrow (+, -, or 0) indicates the observed net outcome to the receiver. The η_p^2 values used to construct this diagram are placed next to the arrows (with superscripts to indicate their source ANOVA table), and are in bold when significant. The strong effects of wasps were hypothesized based on observational data.


Ants
(0)
Wasps
7
(0?)
(+?)
(0)
(–)
8
Herbivores
9
.01^2a
.44^2a
5
(–)
(+)
3+10
.15^2j
(– or 0)
(0)
2+11
.38^2f
Plant
(–)
.71^2a
1
(0)
12
.01^2a
Light environment (shading)

Appendix A List of herbivores, natural enemies, and other plant visitors found on *P. biflora* at the Wilson Botanical Garden, Costa Rica. Question marks indicate uncertain identifications; numbers in parentheses indicate the number of morphospecies that were observed. Notes about Hymenoptera are from Hanson & Gauld (1995) or based on personal observations (G.C. Ward). Flies (Diptera) and spiders (Araneae) were also seen on the experimental plants, but none were collected. Voucher specimens have been deposited at the *Instituto Nacional de Biodiversidad* (INBio) in Santo Domingo de Heredia, Costa Rica.

a. Confirmed and probable herbivores of P. biflora:

Family	***Subfamily***	**Species**	**Notes**
Acrididae	–	spp.	Grasshoppers; rare; observed consuming older leaves only.
Aleyrodidae	–	sp.	Whitefly "pupae" and adults; pupae were found on older leaves only.
Chrysomelidae?	–	spp.?	Unidentified larvae, possibly Alticinae.
Chrysomelidae	Alticinae	*Asphaera nobilitata*	Flea beetle.
Chrysomelidae	Alticinae	*Macroaltica* sp.	Flea beetle, metallic blue, large.
Chrysomelidae	Alticinae	*Monomacra/Resistenciana ornate*	Flea beetle, red and black.
Chrysomelidae	Alticinae	*Monomacra*? sp.	Flea beetle, yellow.
Chrysomelidae	Alticinae	*Omphorita* sp.	Flea beetle.
Chrysomelidae	Alticinae	*Parchicola*? *violacea*?	Flea beetle, metallic blue.
Chrysomelidae	Alticinae	spp. (ca. 10)	Flea beetles; uncommon spp.
Chrysomelidae	Galerucinae	spp. (2)	Leaf beetles.
Curculionidae	Brachyderinae?	sp.	Weevil, verdigris, large; rare.
Gracillariidae	–	sp.	Leaf miner.
Limacidae	–	*Deroceras leavi*?	Slug; rare.
Nymphalidae	Heliconiinae	*Dione juno*	Butterfly; observed adult oviposition and caterpillars.
Nymphalidae	Heliconiinae	*Dryadula praetusa*	Butterfly; observed adult oviposition.
Nymphalidae	Heliconiinae	*Dryas iulia*	Butterfly; observed adult oviposition and caterpillars.
Nymphalidae	Heliconiinae	*Heliconius charitonius*	Butterfly; observed adult oviposition and caterpillars.
Nymphalidae	Heliconiinae	*H. erato*	Butterfly; observed adult oviposition.
Nymphalidae	Heliconiinae	*H. hecalesia*	Butterfly; observed adult oviposition and caterpillars.

b. Confirmed and possible parasitoids of P. biflora herbivores:

Family	***Subfamily***	**Species**	**Notes**
Braconidae	Opiinae	*Opius* spp. (2)	Wasps; parasitoids of Agromyzidae (Diptera); one sp. emerged from a leaf miner on *P. biflora*.
Chalcididae	Chalcidinae	*Conura* spp. (5)	Wasps; parasitoids of Lepidoptera, Chrysomelidae, and other parasitoids; observed feeding from *P. biflora* extrafloral nectaries; one sp. emerged from *H. hecalesia* pupae on *P. biflora*.
Elasmidae	–	*Elasmus* sp.	Wasp; mostly parasitoids of Lepidoptera; emerged from leaf miner on *P. biflora*.
Encyrtidae	–	sp.	Wasp; parasitoids of many host groups; observed sp. was ca. 1 mm long.
Eulophidae	–	sp.	Wasp; parasitoids of many host groups; observed sp. was ca. 1 mm long.
Eulophidae	Eulophinae	sp.	Wasp; parasitoids of insect larvae concealed in leaf tissue.
Eulophidae	Tetrastichinae	sp.	Wasp; parasitoids and predators of many host groups; emerged from a leaf miner on *P. biflora*; observed sp. was ca. 1 mm long.
Eurytomidae	–	sp.	Wasp; parasitoids; emerged from a leaf miner on *P. biflora*, ca. 1 mm long.
Scelionidae	Teleasinae	sp.	Wasp; parasitoids of Carabidae (Coleoptera); observed sp. was ca. 1 mm long.
Scelionidae	Telenominae	*Telenomus* sp.	Wasp; parasitoids of Lepidoptera; emerged from black-colored Heliconiinae eggs on *P. biflora*, ca. 1 mm long.

Appendix A *continued*

c. Confirmed and possible predators of P. biflora herbivores:

Family	***Subfamily***	***Species***	**Notes**
Formicidae	Dolichoderinae	*Dorymyrmex* spp. (2)	Ants; observed feeding from *P. biflora* extrafloral nectaries.
Formicidae	Dolichoderinae	*Tapinoma* sp.	Ant; observed feeding from *P. biflora* extrafloral nectaries.
Formicidae	Formicinae	*Camponotus* sp.	Ant; observed feeding from *P. biflora* extrafloral nectaries.
Formicidae	Myrmicinae	*Crematogaster* sp.	Ant; observed feeding from *P. biflora* extrafloral nectaries.
Formicidae	Myrmicinae	*Pheidole* spp. (5)	Ants; observed feeding from *P. biflora* extrafloral nectaries.
Formicidae	Pseudomyrmecinae	*Pseudomyrmex* sp.	Ant; observed feeding from *P. biflora* extrafloral nectaries.
Ichneumonidae	Cremastinae	*Ptilobaptus*? sp.	Wasp; predators of Lepidopteran larvae; observed feeding from *P. biflora* extrafloral nectaries.
Sphecidae	–	sp.	Wasp; predators of Lepidopteran larvae.
Vespidae	Polistinae	*Agelaia* sp.	Wasp; predators of Lepidopteran larvae.
Vespidae	Polistinae	*Polybia* spp. (2)	Wasps; predators of Lepidopteran larvae; observed feeding from *P. biflora* extrafloral nectaries and attacking heliconiine caterpillars.

d. Other insects seen on P. biflora, possibly only visiting:

Family	***Subfamily***	***Species***	**Notes**
Chrysomelidae	Eumolpinae	*Pseudocloris* sp.	Beetle, metallic brown.
Chrysomelidae	Eumolpinae	spp. (5)	Beetles.
Curculionidae	Prionomerinae?	sp.	Weevil, small; rare; observed feeding from extrafloral nectaries.
Diapriidae	Diapriinae	*Monelata* sp.	Wasp; observed sp. was ca. 1 mm long.
Figitidae	Figitinae	*Neralsia*? sp.	Wasp; parasitoids of Diptera.
Mymaridae	–	sp.	Wasp; parasitoids of insect eggs, but not Lepidoptera.
Scelionidae	Scelioninae	spp. (2)	Wasps; egg parasitoids of Hemiptera; observed spp. were ca. 1 mm long.

CHAPTER 3

BODYGUARD PROTECTION IS NOT REDUCED BY SHADING OF *PASSIFLORA BIFLORA* AT A MID-ELEVATION SITE IN COSTA RICA[1]

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[1] Ward, G. C. and Peterson, C. J. Submitted to *Journal of Tropical Ecology*, April 2002.

ABSTRACT. Plants with extrafloral nectaries are often protected by ant bodyguards, but it is largely unknown to what extent the degree of bodyguard protection is altered by variation in plant resource environment. To test the hypothesis of environmental conditionality of ant defense, ants were experimentally excluded from *Passiflora biflora* (Passifloraceae) plants growing under full sun and two levels of artificial shading (50% and 90%) at a mid-elevation site in southern Costa Rica. The effects of ant exclusion and shading were monitored for 4 mo. As expected, shading dramatically reduced plant growth, flowering, and leaf toughness. Flea beetles (Coleoptera: Chrysomelidae) and Heliconiinae butterfly caterpillars (Lepidoptera: Nymphalidae) tended to be most abundant on the plants in full sun, but butterfly oviposition was not influenced by plant light availability. In contrast, leaf miners (Lepidoptera: Gracillariidae) and whitefly "pupae" (Homoptera: Aleyrodidae) were most abundant on the plants in 50% shade. Ant exclusion, however, showed no effect on any aspect of plant performance or herbivory, probably because of the general scarcity of ants at the study site. Wasp parasitoids and predators were also observed visiting the extrafloral nectaries, and shading had little effect on their abundance or that of ants, despite the major changes in plant characteristics. But in contrast to ants, wasps were very effective plant protectors against heliconiine caterpillars, and it is likely that in their absence *P. biflora* would be decimated by these herbivores. Overall, despite the large differences in plant characteristics and possibly nectar availability, little evidence was found to support shade-induced conditionality of bodyguard defense. Potential reasons for the lack of conditionality are discussed.

KEY WORDS: bodyguard-mediated defense, Costa Rica, extrafloral nectaries, herbivory, light environment, *Passiflora biflora*, tropical premontane rain forest.

INTRODUCTION

The bodyguard role of ants attracted to plants with extrafloral nectaries (EFNs) has been demonstrated experimentally (reviewed by Beattie 1985, Bentley 1977a, Bronstein 1998, Keeler 1989, Koptur 1992), but the degree of plant benefit is highly variable, both within and among species and across environments (Bronstein 1998, de la Fuente & Marquis 1999). In some situations, plants cannot survive without their ant bodyguards (e.g., Janzen 1966), whereas in others, plants gain no apparent benefit from their extrafloral nectar secretions (e.g., Gaume & McKey 1999, Heads & Lawton 1984, Janzen 1975, O'Dowd & Catchpole 1983, Rashbrook *et al.* 1992, Tempel 1983, Whalen & Mackay 1988, Zachariades & Midgley 1999). Some of this variation in benefit may be explained by differences in ant visitation and by the effectiveness of the ant defenders (e.g., Boecklen 1984, Horvitz & Schemske 1984, Smiley 1986). Also, if herbivore attack is low, plants will experience little benefit regardless of how many or what kind of ants are present (Barton 1986, Koptur & Lawton 1988, Rashbrook *et al.* 1992). However, although the importance of environmental "conditionality" in explaining variation in interspecific interactions is well-recognized (Belt 1874, Bronstein 1994a, Cushman 1991, Cushman & Addicott 1991, Keeler 1981, Thompson 1988), it is largely unknown to what extent the variation in bodyguard protection is attributable to variation in the abiotic environment (Bronstein 1998, but see Folgarait & Davidson 1994, 1995).

To investigate the influence of the abiotic environment on bodyguard defense, we conducted ant exclusion experiments with *Passiflora biflora*, a Neotropical vine with EFNs, across three levels of light availability. This study represents an extension of our research on the tritrophic responses to shading of *P. biflora* (Ward & Peterson, unpubl. manuscript). We investigated the effects of light because it is one of the most variable abiotic conditions faced by plants in tropical forests (Chazdon *et al.* 1996, Clark *et al.* 1996), and because its availability may influence extrafloral nectar production, and therefore bodyguard protection. Studies have found that *floral* nectar production and composition is strongly influenced by light intensity (Boose

1997, Michaud 1990, Pleasants 1983, Pleasants & Chaplin 1983, Rathcke 1992, Southwick 1984), and that plants with greater floral nectar rewards receive more pollinator visits and improved pollination (e.g., Real & Rathcke 1991, Thomson *et al.* 1988, Zimmerman 1988), thus it is reasonable to expect light intensity to have similar effects on *extrafloral* nectar production and bodyguard protection. Therefore, we predicted that some of the observed variation in plant performance across different levels of light availability may be due to the indirect effects of light availability on the interactions between insect herbivores and plant bodyguards. Clearly, variation in plant performance across light levels may be a function of environmental effects unrelated to bodyguard defense (e.g., differences in plant physiological response, plant chemical defense, or herbivore attack risk), but the focus of this study was on the contribution of differential plant protection by bodyguards.

A priori, we expected that because extrafloral nectar is mostly carbon-based (Bentley 1977a, Koptur 1992), its production should have lower fitness costs for plants growing in full sun than for plants growing in shaded conditions, where photosynthates are much less likely to be available in excess of the plant requirements for maintenance and growth (Bryant *et al.* 1983, Herms & Mattson 1992). Therefore, we hypothesized that the availability of extrafloral nectar would be decreased by shading—because (a) shaded plants have fewer EFNs overall due to their fewer leaves, (b) shaded plants have fewer EFNs per leaf, or (c) shading reduces nectar production by individual EFNs. In addition, we hypothesized that because of the decreased nectar availability, shaded plants would have fewer plant bodyguards than plants in full sun, and correspondingly greater herbivore abundance and herbivory, thereby resulting in diminished plant performance beyond that of the direct effects of shading.

In this paper, we address the following questions: (1) Do shaded plants suffer more or less herbivore damage than unshaded plants? (2) Do plant bodyguards protect *P. biflora* from insect herbivory? And if so, (3) does shading alter bodyguard protection? It is important to note here that this study was explicitly designed to test for a role of ants as plant protectors, but in contrast to our expectations, we found that wasps at our study site were the main plant bodyguards of *P. biflora*. Therefore, we address the above questions for bodyguards in general, including both ant and non-ant plant protectors.

MATERIALS AND METHODS

Study site

This research was carried out within the grounds of the Robert & Catherine Wilson Botanical Garden in southern Costa Rica (8° 47' N, 82° 57' W; elevation ca. 1100 m), which is part of the Organization for Tropical Studies Las Cruces Biological Station. The Botanical Garden is surrounded by secondary tropical premontane rain forest and abandoned shaded coffee plantation (Hartshorn 1983). Mean annual rainfall is ca. 4 m, with a variable 3-5 month dry season that typically starts in December. The Botanical Garden is maintained by periodic mowing and trimming, but no pesticides are used. The experimental plants (see below) were placed in five sunny locations (i.e., light gaps) within the grounds of the Botanical Garden; four of these groups were located < 10 m from the adjacent secondary forest, and the fifth ca. 50 m away. The ground cover in the selected locations consisted of either mowed grass (two locations) or a thin layer of leaf litter and fine woody debris (three locations). The Botanical Garden was selected as a study site because we felt it represented a reasonable compromise between light availability, biological realism, experimental control, and site security.

Study organisms

Passiflora biflora Lam. (Passifloraceae) is a common herbaceous vine of light gaps, successional forest, and old fields. Leaves are typically bilobed, but leaf shape and size are highly variable. Scattered across the abaxial leaf surface are 2-26 yellow, 1-mm diameter extrafloral nectaries (EFNs), which exude droplets of clear nectar (Apple & Feener 2001; G. C.

W., pers. obs.). The individual plants used in this study were propagated from cuttings taken from wild plants found within a radius of ca. 10 km from the Botanical Garden.

The most commonly seen herbivores on *P. biflora* at the Las Cruces Biological Station were Alticinae flea beetles (Coleoptera: Chrysomelidae), Heliconiinae butterfly caterpillars (Lepidoptera: Nymphalidae), leaf miners (Lepidoptera: Gracillariidae), and whitefly "pupae" (the scale-like sessile nymphal instars; Homoptera: Aleyrodidae). About 16 species of flea beetle were observed on the experimental plants, but only three of these were frequent herbivores (*Monomacra* spp. and *Parchicola* sp.; identified by comparison to specimens at the *Instituto Nacional de Biodiversidad* (INBio), Santo Domingo de Heredia, Costa Rica.). Six species of heliconiine butterflies were observed ovipositing on *P. biflora* at the study site (*Dione juno*, *Dryadula praetusa*, *Dryas iulia*, *Heliconius charitonius*, *H. erato*, and *H. hecalesia*; identified using DeVries 1987), but the caterpillars of two of these species (*Dryadula praetusa* and *H. erato*) were never found on the experimental plants. Using egg morphology, we could not distinguish among Heliconiinae species, except for the distinctive eggs clusters of *Dione juno* (a rare event; see DISCUSSION), thus egg counts represent cross-species totals.

Besides herbivores, we also observed several kinds of potential plant bodyguards on the experimental plants, including ants and wasps (identified using Hanson & Gauld 1995), and web-building spiders (Araneae). The most frequent ants were very small, 1-mm long species (*Dorymyrmex* sp., Dolichoderinae; *Pheidole* sp., Myrmicinae), but occasionally we also observed slightly larger, 3-mm long individuals (*Camponotus* sp., Formicinae; *Pheidole* spp., Myrmicinae). The most frequent wasps included very small, 1-mm long egg parasitoids (*Telenomus* sp., Scelionidae), 4-mm long larval and pupal parasitoids (*Conura* spp., Chalcididae), and 7-mm long generalist predators (*Polybia* spp., Vespidae). We never observed antagonistic interactions between ants and herbivores on *P. biflora*, but we successfully reared *Telenomus* wasps from parasitized heliconiine eggs, *Conura* wasps from parasitized heliconiine pupae, and twice we observed vespids attacking and consuming heliconiine caterpillars. We also successfully reared wasp parasitoids from leaf miners, including *Elasmus* sp. (Elasmidae), Eurytomidae sp., *Opius* sp. (Braconidae), and Tetrastichinae sp. (Eulophidae). The ants and wasps on the experimental plants were frequently observed visiting the EFNs, presumably drinking the secreted nectar; in particular, vespid wasps were commonly observed traplining for extrafloral nectar, moving frequently from leaf to leaf.

Experimental design

At each of the five selected sunny locations, we established groups of six miniature shade houses (or "shade huts"; N = 5 locations x 6 huts = 30), which we constructed from four upright bamboo posts pounded into the ground and then roofed by one of three kinds of covering material (see below), having the overall dimensions of ca. 0.9-m wide x 0.9-m deep x 1.0-m high. At each location, the six shade huts were placed 2-4 m apart, in a staggered arrangement, to prevent shading by neighboring huts. Within each group, covering materials were assigned randomly to the six bamboo structures, such that each group contained two huts made from each of the following: (1) open netting with 1.6 x 1.6 cm mesh (DeerBlock®; Easy Gardener, Inc., Waco, Texas), (2) 50% shade cloth made from knitted black polypropylene (DeWitt Sudden Shade®; purchased from Hummert International, Earth City, Missouri), and (3) 90% shade cloth made from knitted black polypropylene (same source). Regardless of Shade treatment, the covering material was attached only to the sides exposed to direct sunlight (i.e., east, top, and west faces), leaving the two remaining sides (i.e., north and south faces) completely open. This configuration served to intercept direct light, yet permitted free movement of air and access by insects to the enclosed plant (see below). The open netting treatment served as a full sunlight control (0% shade); it inhibited large flying insects (e.g., butterflies) on the same three sides as the knitted shade cloths, but it did not significantly reduce light intensity (see RESULTS: *Effectiveness of treatments*).

In mid-March 1998, we placed one potted *P. biflora* plant within each shade hut, burying the plastic pot to the rim in a central hole. Plant stems were supported using a thin, 1-m tall bamboo pole (with twist-ties). All six plants within each group were derived from the same source plant (five clones in all) to minimize within-group variation due to non-treatment sources. Before placement into the shade huts, the experimental plants were grown for several months in a common garden, and then trimmed to similar stem length (ca. 0.8 m) to reduce inter-plant heterogeneity. Because of this trimming, most plants had very few to no leaves when moved into their randomly assigned shade huts. Immediately after relocation, all plants were sprayed once with 0.001% (w/v) deltamethrin (Decis® 2.5 EC; AgrEvo, Wilmington, Delaware) to remove resident insects. The plants were then watered every 2-3 d for ca. 5 wks, when the dry season ended in mid-April. Throughout the study, the plants were fertilized every 2 wks with a solution of NPK 20-20-20 fertilizer plus micronutrients. No evidence of drought (wilting) or nutrient stress (chlorosis) was observed.

Immediately after being placed into their assigned shade huts, we randomly selected three plants in each group (one of each Shade treatment) to receive a Tanglefoot barrier at the stem base (applied over masking tape). Tanglefoot® Pest Barrier (Tanglefoot Company, Grand Rapids, Michigan) is a sticky non-drying resin used to prevent access to plants by crawling insects. To prevent foraging ants from bypassing this stem barrier, we also applied Tanglefoot to the bases of the bamboo support posts, and hand-trimmed the ground cover within and surrounding the shade hut to ca. 5-cm height. We refreshed the Tanglefoot as necessary.

Light measurement

We quantified the daily photosynthetic photon flux density (PPFD) in the different Shade treatments using eight Line Quantum Sensors (model LI-191SA) cabled to a single data logger (model LI-1000; Li-Cor, Inc., Lincoln, Nebraska). This setup enabled the simultaneous measurement of all six shade huts in each group and at two adjacent positions in full sun. The data logger was configured to take light readings at 5-s intervals and then to record the mean PPFD for every 10-min period. These measurements were taken prior to the installation of the experimental plants (i.e., between November 1997 and February 1998), on days with few clouds, starting at daybreak (5:30 h) and ending at nightfall (17:50 h). Shade hut groups were measured in random order. Half of each light wand (i.e., 50 cm) was covered with two layers of black plastic sheeting to restrict the sensing region to a portion that would fit entirely within the shade hut covering; the PPFD readings were later multiplied by two to correct for this adjustment. Light wands were always oriented on an east-west axis and leveled on wooden stakes above the ground cover to ensure measurement repeatability.

Periodic censuses

From late March to the end of July 1998 (ca. 4 mo.), we periodically quantified the performance of the experimental plants and the abundance of herbivores and potential plant bodyguards present. Specifically, we periodically counted the number of actively growing shoots, leaves, flower buds, open flowers, fruits, heliconiine butterfly eggs, heliconiine caterpillars, beetles, leaves occupied by leaf miners, ants, wasps, and web-building spiders. The approximate date for each census can be determined from the figures or elsewhere below; in all cases, the order of group and plant visitation was randomized.

For the heliconiine butterfly eggs, we recorded egg color (yellow vs. black) to monitor the proportion that were parasitized by scelionid wasps (parasitized heliconiine eggs appear black; Smiley 1978; G. C. W., pers. obs.). In these egg counts (and for caterpillar counts), we did not include observations of the mass-laying *D. juno* (also Heliconiinae) because their occurrence was restricted to a single group near a patch of planted *P. coccinea* Aubl., onto which *D. juno* frequently oviposited (see DISCUSSION).

Ant and wasp counts were conducted during daylight hours, before the afternoon rains. (Ants did not visit plants at night, when it was usually cool and wet.) To quantify wasp abundance, we approached each plant slowly to avoid disturbing the insects present, first counting the larger rapidly moving individuals (usually vespids) from ca. 1-m away, and then moving in closer to count the smaller, more sedentary wasps, usually stationary on the leaf undersides. Each plant was watched for ca. 2 min. during these censuses. Because we were unable to identify most ants and wasps in the field, we used broad morphospecies groups for these arthropod counts. Dipterans were the only major group of plant visitors that we did not quantify in this study because their occurrence seemed to have no direct relationship to the plants (e.g., we never saw them drinking from EFNs), although they may be important prey for the resident spiders. Tachinid flies are known to be natural enemies of butterfly caterpillars (DeVries 1987), but we never encountered these parasitoids during our rearing of heliconiine larvae.

To determine whether shading affects the number of EFNs per leaf, in early April we counted EFNs on a subset of new leaves on each plant (N = 163, 2-12 leaves per plant). We used the average number of EFNs per leaf per plant for the statistical analysis of these data. Although extrafloral nectar was clearly present on most young leaves, we were unable in this study to quantify nectar production by individual nectaries because of its high viscosity.

Leaf harvest

At the end of July 1998, we harvested all leaves from the experimental plants for final measurements. For every leaf harvested (1867 total), we measured leaf area using a moving-belt area meter (model LI-3100; Li-Cor, Inc., Lincoln, Nebraska), and visually estimated herbivore damage using six damage categories (see *Herbivory measurements* below). Leaf toughness was quantified for a random sample (n = 4) of harvested leaves from each plant, using a penetrometer (McCormick Fruit Firmness Pressure Tester, 0-500 g model; McCormick Fruit Tech, Yakima, Washington). Each leaf was perforated 10 times, and the mean values were used in the statistical analysis. We also selected a random sample of mature leaves (n = 10) from each plant to quantify the density of whitefly pupae (the black scale-like nymphs) on the abaxial surface. The mobile first instar nymphs were not counted because of their near invisibility to the naked eye.

Herbivory measurements

We used three distinct methods to quantify the impact of herbivores on the experimental plants.

First, we estimated percent insect damage on all 1867 harvested leaves by visually classifying the damage levels (i.e., the removed area) into the following six categories: 0 – 1%, 1 – 12.5%, 12.5 – 25%, 25 – 50%, 50 – 75%, and 75 – 100%. Then using the following two formulas, we calculated for each leaf the estimated removed area (RA; in cm^2) and the estimated potential area (PA; in cm^2): (1) RA = (AA x PR) / (1 - PR) and (2) PA = AA + RA, where AA is the actual area measured and PR is the midpoint value of the proportion removed. Using these values, we then calculated for each plant the *total percent leaf area removed* (or "total proportion damaged"; Williams & Abbott 1991) by dividing the total RA by the total PA (x 100). This herbivory estimation method is recommend in favor of the "average percent damage" approach when assessing plants with variable leaf sizes (Landsberg 1998, Williams & Abbott 1991). Although the use of damage categories enabled us to quantify herbivory on all leaves, it should be noted that this approach may slightly overestimate actual damage levels (Landsberg 1989).

Second, in early April we tagged all leaves on each plant (*n* = 10 to 52 per plant) with numbered plastic tags (hooked over the petioles), and then at leaf harvest, determined which had survived, to calculate the *percent survival of marked leaves* per plant. Direct observation of herbivores and ants indicated that the leaf tags did not interfere with insect movement. By following the fate of these young fully expanded marked leaves, we were able to quantify leaf disappearance, which the previous method could not capture since it focused on only extant leaves (Coley & Barone 1996).

And third, by counting the number of "empty" leaf nodes (i.e., nodes missing leaves) at the end of the study, we determined the *percent empty leaf nodes* per plant. This method, in contrast to the second method, quantified differences in leaf disappearance for immature and fully expanded leaves. We had noticed early on in this study that most herbivore damage on *P. biflora* occurs when leaves are immature and young, and that when damaged, these leaves would frequently abscise from the plant. In comparison, mature leaves tend not to abscise when damaged, thus we felt that this third method would be useful to quantify herbivore impact on leaves at the earliest stage of development.

For the latter two methods, it is important to recognize that leaves may disappear for reasons unrelated to herbivory (e.g., due to the action of wind or rain, or simply senescence), but we determined in a separate study that *P. biflora* leaves typically persist for longer than four months (i.e., more than the duration of this study) when protected from herbivores (using insecticide), and that leaf abscission rates do not differ between sun and shade when plants are protected (Ward & Peterson, unpubl. manuscript). Thus, we consider the latter two estimates above to be indirect measures of herbivory, primarily of immature and young leaves.

Statistical analysis

The overall experimental layout of this study was a randomized complete block 2 x 3 factorial design: group location was used as a blocking factor (n = 5), and Tanglefoot treatment (open vs. protected) by Shade treatment (0% shade vs. 50% shade vs. 90% shade) were the crossed factors within each location (N = 2 x 3 x 5= 30). For most response variables, we used repeated-measures univariate ANOVA to test for effects of shading and ant exclusion, treating the individual plants as subjects and the census date as the repeated within-subject factor. The blocking factor was not tested (Sokal & Rohlf 1995, p. 348), and the block x treatment interactions were not included in the ANOVA model, thus making the null hypothesis for the among-subject factors that there is no effect of treatment in any block (Newman *et al.* 1997). For the within-subjects tests (e.g., Date, Date x Shade, etc.), the conservative Greenhouse-Geisser estimator was used. When a significant Shade or Date effect was found ($P < 0.05$), we used univariate contrasts based on the estimated marginal means to evaluate the simple effect of Shade at each census date (Winer *et al.* 1991). Because of the scarcity of heliconiine caterpillars (17 total), we used their cumulative number across seven census dates (between 31 March and 15 July) in our analysis, after first determining that in no case were the same caterpillars counted on multiple occasions. These cumulative counts and other non-repeated measure results were analyzed with randomized block one-way or 2 x 3 factorial ANOVA, and the three Shade treatments were compared using Tukey's HSD range test. All data were transformed as necessary to minimize heteroscedasticity, typically using $X' = \log_{10}(X)$ for quantitative measurements, $X' = \sqrt{}(X + 0.5)$ for count data, and $X' = \text{arcsine}\ (\sqrt{}X)$ for proportions (Sokal & Rohlf 1995). All statistical analyses were carried out with *SPSS for Windows*, rel. 10.0 (SPSS, Inc., Chicago, Illinois). Rather than include numerous ANOVA tables, we placed the *P* values of all between-subjects tests in their respective figures or in the text.

RESULTS

Effectiveness of treatments

As expected, the three coverings used to construct the shade huts (0%, 50%, and 90% shade) produced significantly different light environments (Figure 3.1a; $F_{2,23} = 157.9$, $P < 0.0001$), although the actual reductions in total daily PPFD were as follows: 5%, 51%, and 81%, respectively. Light availability under the open netting, however, was not significantly different from that of full sun ($F_{1,14} = 1.14$, $P = 0.303$).

Although the Tanglefoot barriers were 100% effective at excluding ants from the experimental plants ($F_{2,20} = 20.6$, $P = 0.0002$), ants were nevertheless uncommon visitors where they were permitted (e.g., a maximum of 8 ants were seen on any one plant). Secreted nectar droplets were clearly present on the leaves, thus the low abundance of ants occurred despite the apparent availability of extrafloral nectar.

Plant responses

Although *P. biflora* leaves are known to be highly variable in shape, we observed no obvious changes from the typical bilobed form in response to the different Shade treatments. Shaded leaves were noticeably thinner and had significantly lower leaf toughness (Figure 3.1b), but shading had no effect on the number of EFNs per leaf (Figure 3.1c). At one group location, the plants had significantly more EFNs per leaf than did the plants in any other group (mean = 14.5 vs. 10.2; $P < 0.0001$). We believe this difference was likely due to genotypic variation for the trait because each group was populated from cuttings from different source plants.

Shading also significantly reduced the number of leaves (Figure 3.2a) and leaf nodes (extant leaves + empty nodes; Figure 3.2b), although plants in the two shaded treatments differed little in these two characteristics. Total leaf area at leaf harvest, however, was not significantly reduced by shading ($P = 0.152$), probably because shaded plants tended to have larger, thinner leaves. For the first two censuses, shading also resulted in significantly fewer actively growing shoots (Figure 3.2c), but this difference then disappeared when the rainy season started (in mid-April), probably due to recurring shoot damage by insect flea beetles and high variation in shoot number. Plants in 90% shade tended to produce the fewest flower buds (Figure 3.2d), but out of 1180 total buds, we saw only 10 fully developed flowers, all of which occurred on plants in the 0% shade treatment, and only four fruits, three of which were found on shaded plants. In our companion study of *P. biflora* (Ward & Peterson, unpubl. manuscript), the effects of shading were more clear: experimental plants placed in naturally sunny locations produced about 12 fruit per plant, whereas plants placed in shaded locations (equivalent to 91% shade) produced none.

Herbivores and herbivory

Heliconiine butterflies began ovipositing on the experimental plants, usually on actively growing shoots and young leaves, as soon as the plants were placed into the shade huts. Butterflies appeared to have little difficulty navigating around the shade coverings to reach the enclosed plant, but despite large differences in leaf number (Figure 3.2a), heliconiine butterfly oviposition did not significantly differ among the Shade treatments (Figure 3.3a). However, out of 268 total heliconiine eggs, we saw only 17 heliconiine caterpillars during this study, indicating high egg and caterpillar mortality. Although rare, the heliconiine caterpillars were significantly more numerous on the larger plants in the 0% shade treatment (Figure 3.3b), indicating that larval survival may have been slightly lower on the shaded plants. In contrast, herbivorous beetles were frequently observed, and were again significantly more abundant on the plants in 0% shade (Figure 3.3c), although this difference disappeared at the final census. The percent of leaves occupied by leaf miners was also significantly affected by light availability (Figure 3.3e), but in this case, leaf miners occupied the greatest percent of leaves on plants in 50% shade, and this result appeared only on the final two censuses. Similarly, the density of whitefly pupae was also greater on the plants in 50% shade (Figure 3.3d), but here this effect was only apparent when ants were excluded, resulting in a marginally significant Shade x Tanglefoot interaction ($P = 0.0478$).

Because of their rarity and disappearance before reaching the largest, most damaging instar, heliconiine caterpillars had little impact on the experimental plants, except for one incidence of dramatic herbivory by *D. juno* caterpillars (see DISCUSSION). Instead, most leaf and shoot damage was caused by the three most common flea beetles, which typically attacked young leaves and actively growing shoots. If damaged when young, however, *P. biflora* leaves would frequently abscise from the plant, as reflected by the high *percent empty leaf nodes* (mean for each Shade treatment: 0% shade, 55.8%; 50% shade, 55.7%†; 90% shade, 61.3%), but the degree of leaf disappearance did not differ among shade huts ($F_{2,19} = 0 .685$, $P = 0.516^{\dagger}$). In comparison, *total percent leaf area removed* on the extant leaves at the end of the study indicated relatively low levels of tissue loss (means: 0% shade, 5.32%; 50% shade, 4.69%; 90% shade, 3.69%), but again herbivory did not differ significantly among Shade treatments ($F_{2,20} = 1.65$, $P = 0.218$). In contrast, *percent survival of marked leaves* was significantly lower on the most shaded plants (means: 0% shade, 57%; 50% shade, 62%†; 90% shade, 35%; $F_{2,19} = 3.95$, $P = 0.0368^\dagger$), indicating that the marked cohort of leaves on these plants may have experienced higher insect herbivory. († Note: the one plant defoliated by *D. juno* was excluded from these ANOVAs and means.)

Bodyguards and protection

On the plants where ants were permitted, the abundance of ants was unaffected by the shading treatment (Figure 3.4a), despite the large differences in leaf number and possibly extrafloral nectar availability. And despite the effectiveness of the Tanglefoot barrier at keeping out ants, we found no significant effect of ant exclusion on any aspect of plant performance (Figure 3.2) or any measure of herbivory. Ant exclusion also had no significant effect on butterfly oviposition ($P = 0.793$), or the abundance of heliconiine caterpillars ($P = 0.807$), beetles ($P = 0.576$), wasps ($P = 0.807$), and spiders ($P = 0.937$). We observed ants visiting EFNs on the experimental plants, but we never saw antagonistic behavior by ants toward butterfly eggs or other arthropods. Many of the ants seen were small (ca. 1-mm long) and none exhibited aggressive behavior in response to our contact with the plants.

The only evidence for an effect of ant exclusion was revealed in our analysis of whitefly pupae density, as indicated by a marginally significant Shade x Tanglefoot interaction (Figure 3.3d). When the two Tanglefoot treatments were analyzed separately (i.e., open vs. protected), whitefly pupae density was found to be significantly different among Shade treatments only when ants were excluded (shaded bars in Figure 3.3d; $F_{2,8} = 9.0$, $P = 0.009$). These results indicated that the density of whitefly pupae *declined* when ants are excluded (Figure 3.3d), suggesting that ant presence may actually be beneficial to whiteflies, but only on plants growing in full sun.

Besides ants, we also observed wasps and spiders on the experimental plants. In contrast to ants, however, wasps had a substantial impact on *P. biflora* herbivores, having parasitized 38% of the 268 heliconiine eggs by the time they were counted. However, neither wasp abundance (Figure 3.4b) nor percent parasitism of heliconiine eggs (Figure 3.4c) was significantly influenced by the shading treatments. Interestingly, a few weeks after butterfly oviposition reached its lowest level (in late May; Figure 3.3a), egg parasitism also dropped to its lowest (in mid-June; Figure 3.4c), indicating that the activity of wasp parasitoids or parasitism rates may closely track the availability of their egg hosts. On the one date they were counted (16 June), web-building spiders were significantly more abundant on the most shaded plants (mean ± 1 SE: 0% shade, 1.1 ± 0.23; 50% shade, 0.5 ± 0.22; 90% shade, 3.0 ± 0.49; *P* = 0.0001), but we observed no evidence that these spiders served as plant bodyguards (e.g., by capturing flea beetles or heliconiine caterpillars).

DISCUSSION

Environmental conditionality is rejected

We initially hypothesized that shaded plants would be less well defended by ants compared to plants in full sun because the decreased photosynthesis in the shade would result in lower production of extrafloral nectar, at the plant, leaf, or nectary level. And, because shaded plants are less defended, we hypothesized that herbivore abundance and herbivory would be greater on these plants, resulting in their overall reduced plant performance. Therefore, based on these predictions, we expected that plants in full sun would suffer more (in terms of herbivory) by the exclusion of ants than would shaded plants. Yet despite the clear changes in plant performance among Shade treatments, and possibly differences in whole-plant availability of extrafloral nectar, the abundance of ants (and wasps) on *P. biflora* did not respond significantly to differences in light availability, and the exclusion of ants did not reduce herbivory or increase plant performance, regardless of light environment. Consequently, we conclude that environmental conditionality of nectar production was not an important contributing factor to growth and reproduction of *P. biflora* at the study site. However, because ants were scarce on the experimental plants, the potential protective role of ants on *P. biflora* and the environmental conditionality of their plant defense remain to be determined for habitats where ants are abundant plant visitors (e.g., in lowland rain forest; Apple & Feener 2001, Smiley 1978).

Other studies of plants with EFNs have found an effect of the light environment on ant abundance and defense (Bentley 1976, de la Fuente & Marquis 1999), and a significant effect of habitat type, which may be related to light availability, on heliconiine egg parasitism (Smiley 1978). But unlike our work, these studies did not experimentally manipulate the availability of light. While such studies are very useful for understanding the broad differences in bodyguard defense among habitats, they may have little power to reveal the actual mechanism of environmental conditionality if it occurs, because many factors other than plant attractiveness to bodyguards may differ between sunny and shaded habitats. In our study, however, it can probably be safely assumed that few environmental differences existed among the Shade treatments, other than light availability, due to the design and layout of our shade huts; for example, within each group location, all plants probably experienced a similar soil nutrient and temperature environment, and had equal access to ants, wasps, and herbivores.

Only a very few other studies have experimentally manipulated environmental conditions to investigate the potential role of the abiotic environment on bodyguard defense. For example, in their extensive greenhouse study of *Cecropia* (Cecropiaceae), Folgarait and Davidson (1994, 1995) investigated the effects of light and nutrient availability on a broad range of plant defenses, including putative chemical, physical, phenological, and bodyguard food rewards. In general, they found that the treatment effects on each defense depended largely on its nutrient composition (Folgarait & Davidson 1995), as predicted by the Carbon/Nutrient Balance hypothesis (Bryant *et al.* 1983, Herms & Mattson 1992). The logical next step for this research would be to conduct a field experiment to determine the ecological relevance of this environmentally-induced variation in herbivore defenses and bodyguard rewards. Similarly, the logical next step for our work with *P. biflora* would be to investigate in a greenhouse setting the physiological effects of shading on the production of extrafloral nectar, to determine for this species whether variation in nectar rewards does in fact occur.

Ant-guard hypothesis is rejected

Based on every measure of plant performance and herbivory, we found no evidence in this study that *P. biflora* benefited from ant visitation. The lack of a beneficial effect of ants was unexpected because ants are often attracted to plants with EFNs, and many ant exclusion experiments have shown that ant visitors can benefit plants by reducing herbivory (reviewed by Beattie 1985, Bentley 1977a, Bronstein 1998, Keeler 1989, Koptur 1992). However, the rejection of the ant-guard hypothesis for plants with EFNs is not unknown (e.g., Gaume & McKey 1999, Heads & Lawton 1984, Janzen 1975, O'Dowd & Catchpole 1983, Rashbrook *et al.* 1992, Tempel 1983, Whalen & Mackay 1988, Zachariades & Midgley 1999), and several hypotheses have been proposed to account for the apparent lack of ant defense. Here we consider four hypotheses for the case of *P. biflora*.

Hypothesis #1: Herbivory is too low for ant defense to be effective (Barton 1986, Boecklen 1984, O'Dowd & Catchpole 1983). This hypothesis is rejected for *P. biflora* because herbivores were observed to cause much damage to the experimental plants (which we have also demonstrated with an insecticide exclusion experiment; Ward & Peterson, unpubl. manuscript).

Hypothesis #2: Herbivores are resistant to ant attack (Heads & Lawton 1985, Koptur & Lawton 1988, Rashbrook *et al.* 1992). We also reject this hypothesis (except possibly for leaf miners; Koptur & Lawton 1988) because ants in general are known to be good defenders against a variety of arthropod herbivores, even when ants are relatively "timid" (Gaume *et al.* 1997, Horvitz & Schemske 1984, Letourneau 1983), as appeared to be the case in this study.

Hypothesis #3: Plants do not attract ant foraging. This could occur, for example, if (a) only older leaves with inactive EFNs were present (O'Dowd 1979), (b) herbivores have destroyed the young leaves with the most active EFNs, or (c) for reasons related to nectar quality (Lanza *et al.* 1993). But we reject this hypothesis because ants were clearly attracted to the experimental plants, because they appeared as soon as the plants were placed into the shade huts (when most plants had very few leaves), and because foraging ants have also been observed on wild *P. biflora* (Apple & Feener 2001, Smiley 1978; G. C. W., pers. obs).

Hypothesis #4: Ant abundance is too low at the site to provide plant protection (Barton 1986, Boecklen 1984, Koptur 1985, Rashbrook *et al.* 1992, Smiley 1986, Zachariades & Midgley 1999). We suggest this last hypothesis provides the most likely explanation for the lack of ant defense on the experimental plants because our ant counts were much lower than those in other tropical studies of EFN plants where ant defense has been experimentally demonstrated (e.g., de la Fuente & Marquis 1999, Janzen 1966, Koptur 1984, Smiley 1986, Whalen & Mackay 1988). We do not believe ant visitation was low on the experimental plants due to the Botanical Garden being inhospitable habitat for ants, because when we tested this possibility by placing honey bait stations adjacent to the experimental plants and in nearby unkempt secondary vegetation, the ant counts in these two locations were not significantly different (G. C. W., unpubl. data). Feener and Schupp (1998) also found little to no difference in ant abundance between nearby forest and light gap locations. At a lowland site in Costa Rica, Apple and Feener (2001) found much higher ant visitation rates on *P. biflora* than did our study, suggesting that the low ant visitation we observed may be a function of higher site elevation. Koptur (1985) found that extrafloral nectar-drinking ants were frequent visitors to *Inga* trees growing at low elevations in Costa Rica, whereas on *Inga* trees at mid-elevations, nectar-drinking ants were rare or absent, being replaced by wasp visitors. In general, ant abundance in several tropical regions has been documented to decline with increasing elevation (e.g., Bentley 1977a, Brühl *et al.* 1999, Olson 1994, Samson *et al.* 1997), and typically, the sharpest decline occurs at the transition between premontane and lower montane rain forest (Olson 1994, Samson *et al.* 1997), which happens to be the approximate location of the Wilson Botanical Garden. Mid- and higher elevations may be inhospitable to ants because the vegetation there is periodically enveloped in clouds, and therefore more continuously wet and cool (Bentley 1977a, Olson 1994).

Although ants were ineffective bodyguards of the experimental plants, we found in this study that ant visitation may at times be slightly detrimental to *P. biflora*, because when ants were excluded from plants in 0% shade, the density of whitefly pupae actually *decreased* (Figure 3.3d). Whitefly nymphs and adults are known to flip away their honeydew excretions (Byrne & Bellows 1991), thus this apparent protection by ants is probably not due to active tending of whiteflies, as occurs with other Homopterans (Way 1963). A more probable explanation may be that ant presence simply interferes with the impact of whitefly predators and parasitoids, and that the apparent protection of whiteflies was only evident in full sun because it was only on these plants that ants were attracted in sufficient number to interfere with whitefly natural enemies.

Wasp-guard hypothesis is supported

Bentley (1977b) also noted low ant abundances in the locality of the Wilson Botanical Garden, and she used this observation to explain the loss of extrafloral nectaries from *Bixa orellana* (Bixaceae) growing in the area. Thus if ants really are uncommon at our study site, why does *P. biflora* growing at the locality continue to produce leaves with EFNs? It seems unlikely that non-functional EFNs would persist in the population over the long term (Keeler 1985), thus EFNs may serve a biological function other than ant attraction. Several alternative hypotheses have been proposed (reviewed by Beattie 1985, Koptur 1992), but we suggest that the function of EFNs at the study site is best explained by a "wasp-guard" hypothesis, i.e., that the EFNs serve to attract wasp parasitoids and predators, not ants, given the tremendous impact of these wasps on heliconiine eggs and larvae. We predict that if these flying bodyguards could be experimentally excluded, as we excluded ants with Tanglefoot, the impact of heliconiine caterpillars on *P. biflora* would be very much greater. Koptur's (1985) observations of *Inga* suggest that ants are the primary plant protectors at low elevations, whereas wasps take over at the higher elevations, which may indicate that these two groups of enemies interfere with each other in the lowlands, but not at higher elevations where ants are scarce. We suggest that a similar phenomenon may hold for the wasp bodyguard defenders of *P. biflora*.

We observed in this study one case of dramatic herbivory that illustrates the potential impact that heliconiine caterpillars can have on *P. biflora*. Midway during the study (25 May), we discovered several clutches of *Dione juno* (Heliconiinae) eggs on each of three plants in one group location (99 and 70 eggs on the 0% shade plants without and with Tanglefoot, respectively, and 38 eggs on the 50% shade plant without Tanglefoot). This particular group happened to be located 20-30 m from a fence covered by *Passiflora coccinea* (a non-native species), where *D. juno* frequently oviposited. On the two 0% shade plants, the numerous gregarious caterpillars that hatched from these eggs caused very little herbivory because the leaves holding the large aggregations of early instar larvae were abscised from the plant, effectively nullifying the herbivore threat because the fallen caterpillars were unable to relocate the plants. However, on the 50% shade plant, a few *D. juno* caterpillars (< 5) escaped leaf abscission, and in < 10 days, these caterpillars consumed all 25 leaves on the plant. By the end of the study, this defoliated plant had more than recovered, but this one herbivory incident resulted in it being barren for 2-3 wks.

It is easy to understand why most investigators have focused on the role of ants as plant protectors, given the conspicuousness of ants on EFN plants and the ease with which ants can be experimentally excluded (e.g., Beattie 1985, Bentley 1977a). However, parasitoids and non-ant predators are also often observed at EFNs (Hespenheide 1985, Keeler 1978, Koptur 1985, 1991, 1992; Koptur & Lawton 1988, Lawton & Heads 1984, Price *et al.* 1980, Ruhren & Handel 1999, Smiley 1978, Stephenson 1982, Tempel 1983, Zachariades & Midgley 1999), yet only a very few studies have examined the potential bodyguard role of these non-ant arthropods (de la Fuente &

Marquis 1999, Koptur 1985, 1991, Pemberton & Lee 1996, Ruhren & Handel 1999, Smiley 1978, Treacy *et al.* 1987). Therefore, we agree with Keeler (1980) that it is premature to extrapolate solely an ant-guard function of EFNs, even in situations where ants are by far the most common plant visitors (e.g., Tempel 1983). Many prior studies may have missed the important contribution of these highly mobile, non-ant bodyguards.

In addition, some researchers have discounted the likelihood that EFNs could evolve to attract wasp bodyguards, even in situations where wasp visitors to EFNs have a major impact on the insect herbivores (e.g., Koptur 1985, 1991). This view was taken because it was assumed that the wasp visitors were parasitoids that allowed their larval hosts to continue to develop and feed, killing them only at the pupal stage (i.e., koinobionts; Hanson & Gauld 1995). Therefore, it was expected that the resulting fitness benefits of wasp visitation would be felt only by neighboring plants, and not by the individual plant bearing the cost of EFNs. However, by recognizing that EFNs may also attract wasp egg parasitoids and larval predators, as we observed on *P. biflora*, the direct fitness benefit to the host plant is easily conceivable due to the immediate lethal impact of these natural enemies.

Effects of shading on herbivores and herbivory

Regardless of the effects of shading on bodyguard defense, the Shade treatments used in this study clearly induced changes in leaf toughness (Figure 3.1b) and perhaps other aspects of leaf quality. Possibly in response to these changes, heliconiine butterflies oviposited on the shaded plants (with softer leaves) as often as on the plants in full sun (with tougher leaves), even though the shaded plants had poorer growth. Nevertheless, despite the equal oviposition, heliconiine caterpillars appeared to have slightly higher mortality on the shaded plants. Since herbivorous beetles were also most abundant on the plants in full sun, it appears the induced change in leaf toughness, by itself, is insufficient to predict host plant suitability for these specialist herbivores.

The leaf miners and whitefly pupae showed a more complex response to the shade huts, having their greatest abundance on plants in 50% shade (although for whitefly pupae, this observation was true only on the ant-excluded plants). Possibly in 90% shade, the extreme thinness of leaves is insufficient tissue for leaf miner larvae, whereas in full sun, the high leaf toughness may limit their successful establishment (but see Potter 1992). For whiteflies, as phloem-feeders, the plants in 90% shade may have insufficient availability of photosynthates, whereas plants in the full sun may be controlled by wasp predators and parasitoids, not by toughness, as suggested by their apparent positive association with ants (Figure 3.3d). In future work, it may be possible to determine (using a dissecting scope) the degree to which these minute herbivores are attacked by predators and parasitoids, and whether the impact of their natural enemies is altered by shading.

We were unable in this study to quantify shade-induced changes in leaf chemistry (e.g., changes in leaf cyanogenesis), but leaf toughness has been found to be one of the best predictors of insect herbivory (Coley 1983). Therefore our observation that the most shaded plants, with the lowest leaf toughness, had the lowest *percent survival of marked leaves* is consistent with what has been found in other tropical systems (Coley 1983). Herbivores were in fact less abundant on these plants, thus it is more likely that the greater loss of marked leaves was due to greater per capita herbivore impact or possibly lower plant tolerance to insect damage. However, for our two more comprehensive measures of herbivory (i.e., *total percent leaf area removed* and *percent empty leaf nodes*), we detected no effect of shading. This inconsistency may have occurred because the marked-leaf method used only a single cohort of leaves produced at the beginning of the study, and the leaves or herbivore impact may have changed during the course of this work. It is unlikely that the observed lower survival in 90% shade was due to differences in exposure to abiotic forces (e.g., wind and rain), because if this was the case, leaf survival should have been lowest on the plants under open netting, not 90% shade cloth.

In addition, nearly all herbivory that occurred on the experimental plants was caused by genus-restricted specialists (primarily flea beetles) because generalist herbivores (e.g., grasshoppers) were almost never seen. This result is consistent with the observation that specialist herbivores cause far more damage in tropical forests than do generalists (Barone 1998). We also observed that the majority of insect herbivory on *P. biflora* occurs to developing leaves and shoots, whereas mature leaves were relatively immune, which is also typical for tropical forest plants (Coley 1983, Coley & Barone 1996). Flea beetles may have caused the most damage on *P. biflora* because their protective elytra and jumping behavior may make them relatively resistant to the wasp bodyguards that were present. Thus, it may be possible to generalize that EFN function is primarily to attract protectors against heliconiine caterpillars, whereas *Passiflora* leaf toughness and cyanogenesis (Spencer 1988) function primarily as defenses against flea beetles. If this is the case, the known diversity of cyanogenic compounds in *Passiflora* may be largely the result of an "arms race" with flea beetles, not solely heliconiine caterpillars as is usually assumed (e.g., Spencer 1988). However, compared to Heliconiinae, the ecology of flea beetles on *Passiflora* has been very little studied.

Plant defenses and the Carbon/Nutrient Balance hypothesis

As mentioned above, we were unable in this study to quantify shade-induced changes in extrafloral nectar production or leaf cyanogenesis, which in addition to leaf toughness, are probably the most important anti-herbivore defenses of many *Passiflora* species (Spencer 1988). We do know that the *P. biflora* plants used in this study were cyanogenic, in both sun and shade, and that the degree of cyanogenesis declined greatly with leaf age (G. C. W., unpubl. data). We can also reject the possibility that shading affects extrafloral nectar production by altering the number of EFNs per leaf (Figure 3.1c). In addition, our results suggest that on a whole-plant basis, shading may have caused an overall decrease in extrafloral nectar production because significantly fewer leaves were produced by the shaded plants (Figure 3.2a). However, other than these observations and the effects of shading on leaf toughness (Figure 3.1b), the particular effects of shading on *P. biflora* chemical and bodyguard defenses are unknown.

However, by reference to the Carbon/Nutrient Balance (CNB) hypothesis (Bryant *et al.* 1983, Herms & Mattson 1992), we can make several specific predictions about *P. biflora* defenses. These predictions are shown graphically in Table 3.1. Plant growth is expected to be greatest under conditions where neither light nor nutrient availability is limiting (Table 3.1, lower right corner), and to be poorest where both light and nutrients are limiting (Table 3.1, upper left corner). But when light and nutrient availability is not balanced, low soil nutrient availability is expected to limit growth more than is photosynthesis, due to physiological trade-offs, such that photosynthetically-fixed carbon will be accumulated in excess of growth requirements, and thereby be available for allocation to carbon-based defenses (Table 3.1, lower left corner). In contrast, low light availability is expected to limit photosynthesis and growth more than nutrient absorption, such that the nutrients assimilated in excess of growth requirements will be available for allocation to nutrient-based (e.g., nitrogen-based) defenses (Table 3.1 upper right corner). The actual allocation of excess resources to defenses, however, will depend on the relative fitness benefits compared to alternative uses (e.g., storage; Herms & Mattson 1992). Thus, when herbivory risk is high, plant allocation to carbon-based defenses (e.g., extrafloral nectar) is expected to be greatest under conditions of high light and low nutrient availability, and to be lowest under conditions of low light and high nutrient availability, whereas plant allocation to cyanogens (and other nitrogen-based defenses) is expected to show the reverse pattern.

As illustrated in Table 3.1, the predictions of the CNB hypothesis hinge on the *relative* availability of light and soil nutrients. In our study, we did not measure soil fertility, but we suggest that nutrient availability was probably at an intermediate level because experimental plants were periodically watered with a fertilizer solution. Therefore, based on the above predictions, the experimental plants in the 0% shade treatment were probably the most defended by extrafloral nectar (Table 3.1, bottom center), and the plants in the 90% shade treatment were probably the most defended by cyanogens (Table 3.1, top center). Although the CNB hypothesis is generally supported (Herms & Mattson 1992, Koricheva *et al.* 1998, but see Hamilton *et al.* 2001), we know of no studies that have examined the combined influence of light and nutrient availability on extrafloral nectar production or leaf cyanogenesis.

Although our study was designed to investigate changes in bodyguard defense at the whole-plant level, it may be useful to understand the particular scale at which environmental plasticity actually occurs (i.e., at the plant, leaf, or individual nectary level) and how this influences the plant's attractiveness to potential bodyguards. For example, if plasticity only occurs at the whole-plant level (i.e., plants are distinguished by number of leaves, not by changes in nectar secretion per nectary), then shade-induced variation in extrafloral nectar availability may have little effect on visitation by egg parasitoids because these tiny wasps generally feed from only a few nectaries per plant; but for larger traplining vespids, which visit numerous nectaries per plant, such plant-to-plant variation may greatly alter plant attractiveness. Moreover, the scale at which environmental plasticity occurs may be useful to understand the sensitivity of extrafloral nectar production to variable light conditions. For example, if plasticity occurs at the nectary level (and thereby also at the whole-plant level), then we might expect that under low nutrient conditions, increasing light availability will rapidly result in greater extrafloral nectar production *per nectary*, whereas nectar production *per plant* (i.e., summed across all leaves) will lag slightly behind, because the former plant response is less limited by nitrogen availability than the latter (Table 3.1).

Conclusions

Based on the observed differences in growth across Shade treatments, our results are consistent with observations of greater abundance of wild *P. biflora* in sunny habitats (Schupp & Feener 1991, Smiley 1978; G. C. W., pers. obs.). We found that specialist herbivores, particularly flea beetles, have a large impact on the performance *P. biflora*, and it appears that the abundance of most herbivores was influenced by light-induced changes in plant characteristics. Nevertheless, using three measures of herbivory, we found little evidence that the degree of insect herbivory was influenced by the experimental shading. Although extrafloral nectar was clearly available on the experimental plants, we also found no evidence that the ants attracted to this food reward were protecting *P. biflora* from herbivory, or that the degree of bodyguard protection by ants was altered by the plant's light environment. We conclude that low ant density at the mid-elevation study site was the primary factor limiting the occurrence of ant defense of *P. biflora*, but we hypothesize that the EFNs are still favored at this elevation because they continue to attract non-ant bodyguards, such as wasps, which played an important role in protection against heliconiine caterpillars, but not flea beetles. Although we did not directly manipulate wasp abundance on the experimental plants, this study provides one of the first demonstrations of the important bodyguard role of wasp parasitoids and predators. Therefore, the habitat preferences of *P. biflora* appear to be determined largely by its photosynthetic response to light availability and possibly greater impact by herbivores in the shade, but not by differential bodyguard defense. Further inquiry is needed to determine whether *P. biflora* benefits from ant visitation at lower elevations, where ant densities on *P. biflora* are much higher (Apple & Feener 2001).

In the last two decades, biologists have substantially improved our understanding of the direct effects of light availability on photosynthesis and growth (reviewed by Chazdon *et al.* 1996), yet relatively few studies have attempted to understand how light heterogeneity may affect biotic interactions between plants, herbivores, and their natural enemies. Most previous studies of plants with bodyguard rewards have viewed these traits as static life history attributes (Bronstein 1994b, but see Agrawal & Rutter 1998, Folgarait & Davidson 1994, 1995; Koptur 1989, Stephenson 1982). Although we found little evidence for light-induced conditionality in ant or wasp defense of *P. biflora*, we suggest that this topic is worthy of further investigation in other habitats and with different plant species. Given the commonness of extrafloral nectaries, occurring in at least 93 plant families and 332 genera from both tropical and temperate habitats

(Koptur 1992), we believe there remains great potential to explain variation in plant-protector mutualistic interactions via consideration of the abiotic environment. We suggest that environmental conditionality may be an important contributor to the observed spatial and temporal variation in plant bodyguard defenses, and that an improved understanding of environmental conditionality is of general importance to studies of tritrophic interactions (Price *et al.* 1980, Thompson 1988).

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Table 3.1. Expected patterns of growth and defense for plants in a variety of hypothetical light and soil nutrient environments, based on the Carbon/Nutrient Balance hypothesis (Bryant *et al.* 1983). For each of the nine combinations below, the expected growth response is shown in the upper left corner, the expected allocation to carbon-based defense is shown in the center, and the expected allocation to nitrogen-based defense is shown in the lower right corner. Symbols are as follows: "0" = no growth or allocation to defense, "+" = low levels, "++" = medium levels, and "+++" = high levels. For carbon-based defense, the expected allocation is expressed both in terms of leaf concentration and (in parentheses) extrafloral nectar volume (see footnotes). Thus, plants growing in high-light, low-nutrient environments are likely to have high allocation to extrafloral nectar (a carbon-based defense), whereas plants growing in low-light or high-nutrient environments are likely to have low allocation to extrafloral nectar. See text for additional details.

	NUTRIENT AVAILABILITY		
LIGHT AVAILABILITY	Low	Intermediate	High
Low	0/+ 0 0	+ 0 ++	+ 0 +++
Intermediate	+ ++ (+[a], ++[b]) 0	++ 0 0	++ 0 ++
High	+ +++ (++[a], +++[b]) 0	++ ++ (++[a], ++[b]) 0	+++ 0 0

[a] Expected production of extrafloral nectar *per plant*.

[b] Expected production of extrafloral nectar *per nectary*.

Figure 3.1. (a) Total daily light availability (PPFD, photosynthetic photon flux density) within the three types of shade huts, (b) effect of these Shade treatments on leaf toughness, and (c) on average number of extrafloral nectaries per leaf (*n* = 10 for each bar). Numbers reported in each graph are *P* values from randomized block one-way ANOVA for the effect of shading (indicated by "S"). Different letters above error bars indicate significant differences ($P \leq 0.05$) among Shade treatments, based on Tukey's HSD range test.

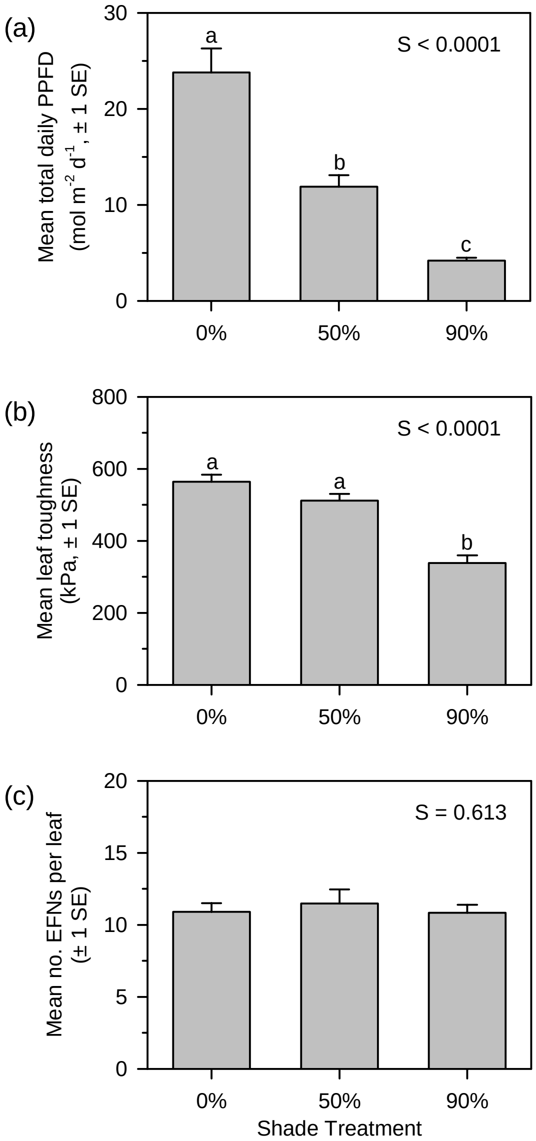

(a)
Mean total daily PPFD (mol m^{-2} d^{-1}, ± 1 SE)
30
20
10
0
S < 0.0001
a
b
c
0%
50%
90%
(b)
Mean leaf toughness (kPa, ± 1 SE)
800
600
400
200
0
S < 0.0001
a
a
b
0%
50%
90%
(c)
Mean no. EFNs per leaf (± 1 SE)
20
15
10
5
0
S = 0.613
0%
50%
90%
Shade Treatment

Figure 3.2. Comparison of plant growth and reproduction in the three Shade treatments: (a) number of leaves, (b) number of leaf nodes, (c) number of shoots, and (d) number of flowers buds. In all graphs, *n* = 10 for each line. To improve clarity, only the combined results for plants with and without Tanglefoot are shown because in no case did Tanglefoot have a significant treatment effect. Numbers reported in each graph are the *P* values from repeated-measures univariate ANOVA for the effects of Shade ("S"), Tanglefoot ("T"), Shade x Tanglefoot interaction ("S x T"), and census date ("D"). The results for other within-subject interaction tests are not shown, unless found to be significant ($P \leq 0.05$). Asterisks are used to indicate census dates when significant simple effects of shading occurred (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

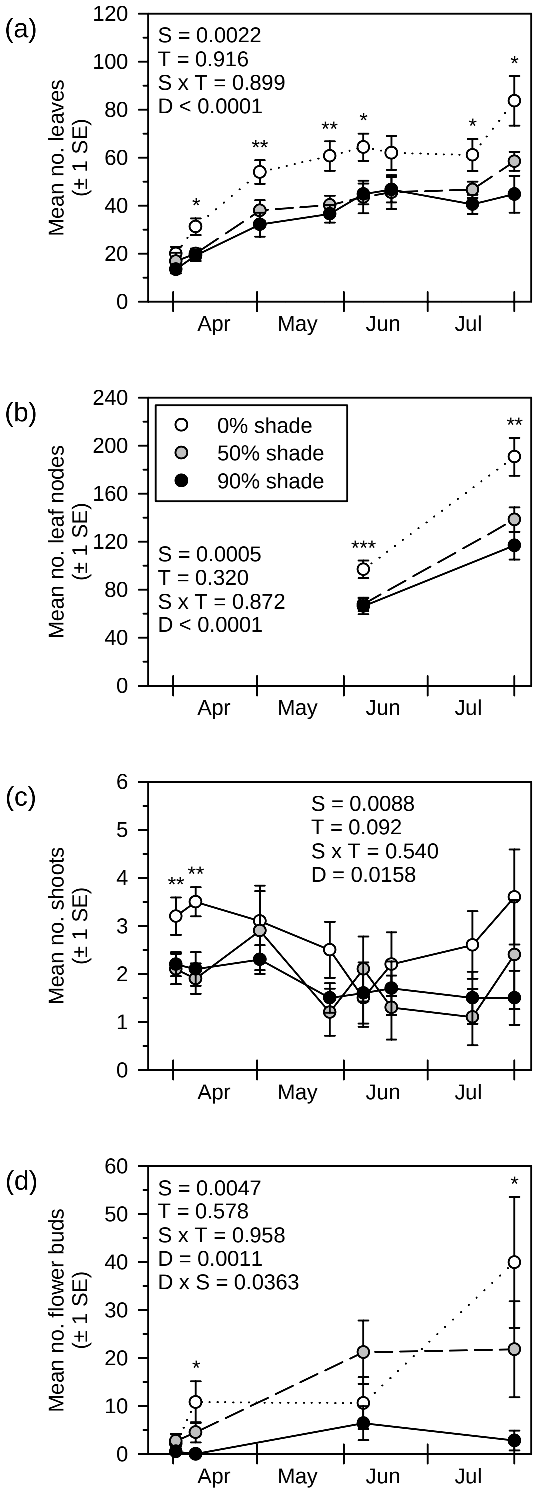

(a)
S = 0.0022
T = 0.916
S x T = 0.899
D < 0.0001
Mean no. leaves (± 1 SE)
0
20
40
60
80
100
120
*
**
**
*
*
*
Apr
May
Jun
Jul
(b)
0% shade
50% shade
90% shade
S = 0.0005
T = 0.320
S x T = 0.872
D < 0.0001
Mean no. leaf nodes (± 1 SE)
0
40
80
120
160
200
240
***
**
Apr
May
Jun
Jul
(c)
S = 0.0088
T = 0.092
S x T = 0.540
D = 0.0158
Mean no. shoots (± 1 SE)
0
1
2
3
4
5
6
**
**
Apr
May
Jun
Jul
(d)
S = 0.0047
T = 0.578
S x T = 0.958
D = 0.0011
D x S = 0.0363
Mean no. flower buds (± 1 SE)
0
10
20
30
40
50
60
*
*
Apr
May
Jun
Jul

Figure 3.3. Comparison of herbivore abundances in the three Shade treatments: (a) number of heliconiine eggs, (b) number of heliconiine caterpillars, (c) number of beetles, (d) whitefly pupae density, and (e) percent of leaves occupied by leaf miners. In (a), (c), and (e), *n* = 10 for each line; in (b) and (d), *n* = 5 for each bar. In graphs (a), (c), and (e), only the combined results for plants with and without Tanglefoot are shown because in no case did Tanglefoot have a significant treatment effect. Numbers reported in these graphs are the *P* values from repeated-measures univariate ANOVA for the effects of Shade ("S"), Tanglefoot ("T"), Shade x Tanglefoot interaction ("S x T"), and census date ("D"); the results for other within-subject interaction tests are not shown because none were significant ($P \leq 0.05$). Asterisks are used to indicate census dates when significant simple effects of shading occurred (* $P \leq 0.05$, ** $P \leq 0.01$). In graphs (b) and (d), numbers are the *P* values from randomized block 2 x 3 factorial ANOVA. In (b), different letters above error bars indicate significant differences ($P \leq 0.05$) among Shade treatments for the two Tanglefoot treatments combined (based on Tukey's HSD range test). In (d), because of a significant S x T interaction, capital and lowercase letters are used to indicate separate comparisons of the Shade treatments for each Tanglefoot treatment.

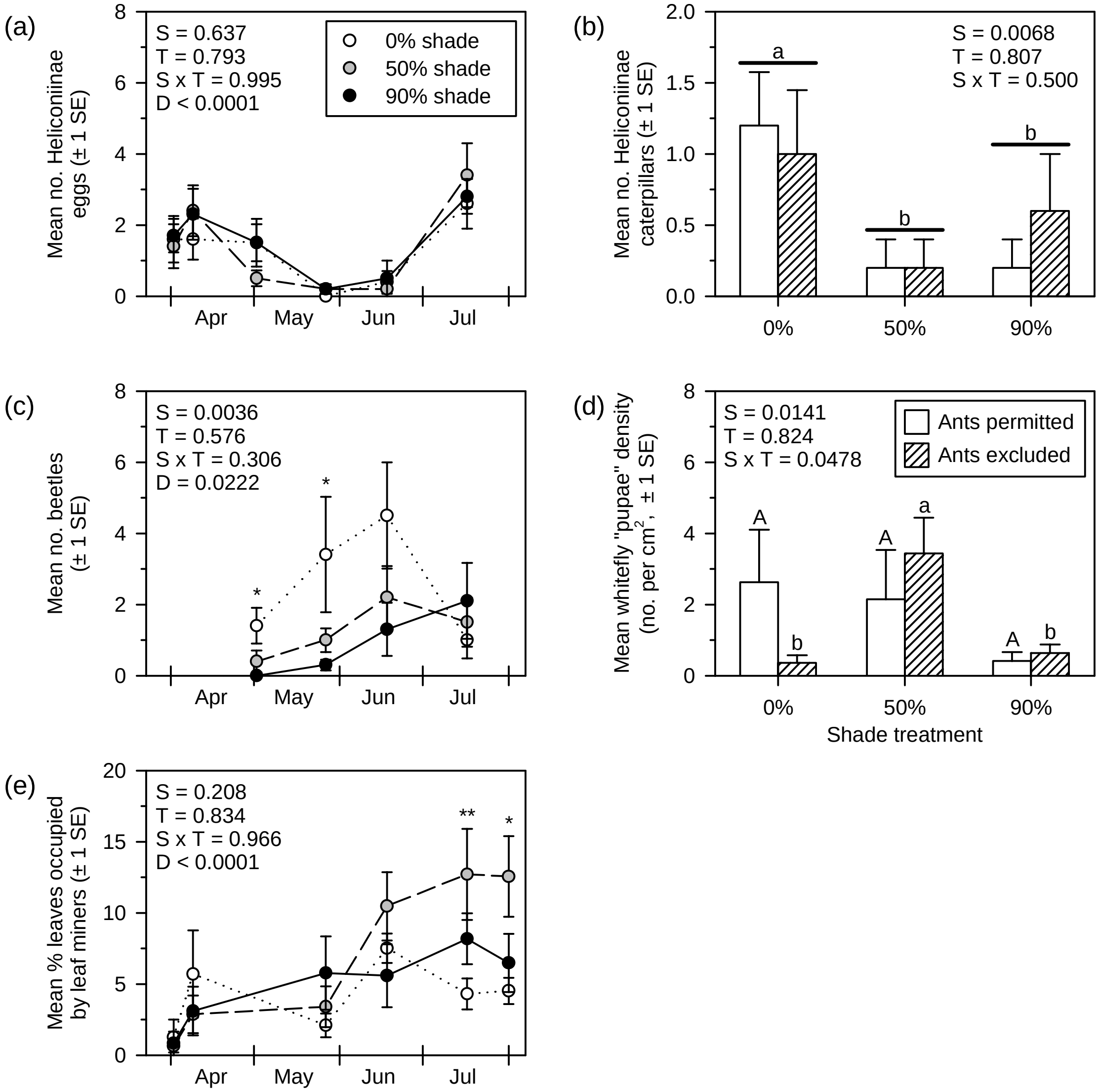
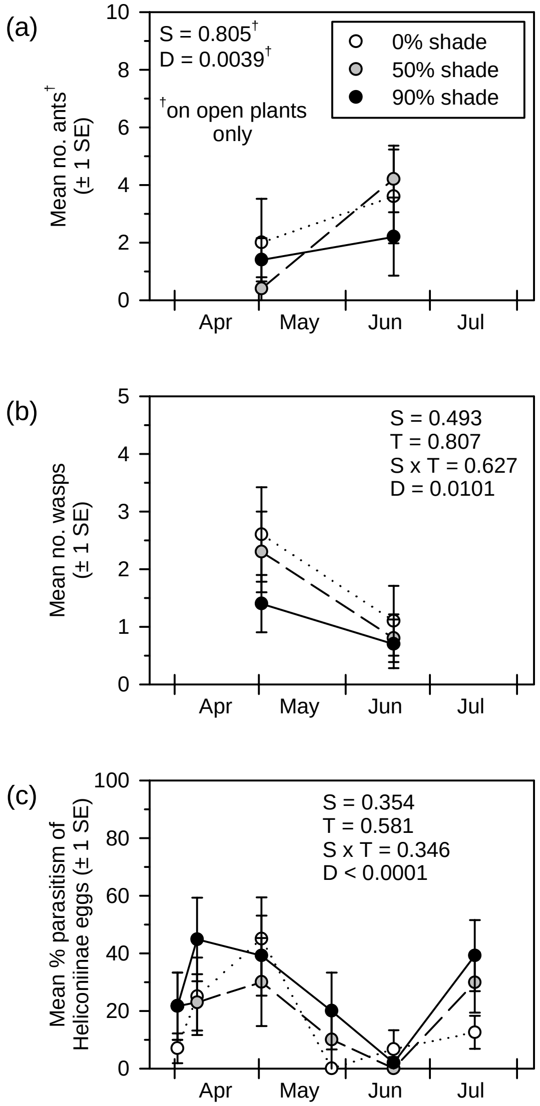

(a)
Mean no. Heliconiinae eggs (± 1 SE)
S = 0.637
T = 0.793
S x T = 0.995
D < 0.0001
○ 0% shade
● 50% shade
● 90% shade
0
2
4
6
8
Apr
May
Jun
Jul
(b)
Mean no. Heliconiinae caterpillars (± 1 SE)
S = 0.0068
T = 0.807
S x T = 0.500
a
b
b
0.0
0.5
1.0
1.5
2.0
0%
50%
90%
(c)
Mean no. beetles (± 1 SE)
S = 0.0036
T = 0.576
S x T = 0.306
D = 0.0222
*
*
0
2
4
6
8
Apr
May
Jun
Jul
(d)
Mean whitefly "pupae" density (no. per cm², ± 1 SE)
S = 0.0141
T = 0.824
S x T = 0.0478
☐ Ants permitted
▨ Ants excluded
A
b
A
a
A
b
0
2
4
6
8
0%
50%
90%
Shade treatment
(e)
Mean % leaves occupied by leaf miners (± 1 SE)
S = 0.208
T = 0.834
S x T = 0.966
D < 0.0001
**
*
0
5
10
15
20
Apr
May
Jun
Jul

Figure 3.4. Comparison of bodyguard abundances in the three Shade treatments: (a) number of ants, (b) number of wasps, and (c) percent parasitism of heliconiine eggs. Only the combined results for plants with and without Tanglefoot are shown because in no case did Tanglefoot have a significant treatment effect. Numbers reported in each graph are the P values from repeated-measures univariate ANOVA for the effects of Shade ("S"), Tanglefoot ("T"), Shade x Tanglefoot interaction ("S x T"), and census date ("D"). The results for other within-subject interaction tests are not shown because none were significant ($P \leq 0.05$).


(a)
Mean no. ants† (± 1 SE)
10
8
6
4
2
0
S = 0.805†
D = 0.0039†
†on open plants only
0% shade
50% shade
90% shade
Apr
May
Jun
Jul
(b)
Mean no. wasps (± 1 SE)
5
4
3
2
1
0
S = 0.493
T = 0.807
S x T = 0.627
D = 0.0101
Apr
May
Jun
Jul
(c)
Mean % parasitism of Heliconiinae eggs (± 1 SE)
100
80
60
40
20
0
S = 0.354
T = 0.581
S x T = 0.346
D < 0.0001
Apr
May
Jun
Jul

CHAPTER 4

THE *RAISON D'ÊTRE* OF EXTRAFLORAL NECTARIES: A GENERAL FRAMEWORK FOR THEIR EVOLUTION AND VARIATION[1]

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[1] Ward, G. C. and Peterson, C. J. To be submitted to *Biological Reviews.*

1. INTRODUCTION

Thomas Belt was among the first to deduce the bodyguard role of ants on plants bearing food rewards and that this ant-plant relationship was greatly influenced by the plant's abiotic environment (Belt 1874 [1985]). Specifically, he noticed that when resident ant bodyguards were present on bull-horn acacias growing in savannahs, the plants were never attacked by leaf-cutting ants, but when these same acacias were grown in his forest garden, the leaf-cutting ants would defoliate them, because, as he deduced, the appropriate bodyguard ants were absent. Moreover, Belt noticed that when the bodyguard ants were present,

> "[t]*hese ants seem at first sight to lead the happiest of existences. Protected by their stings, they fear no foe. Habitations full of food are provided for them to commence housekeeping with, and cups of nectar and luscious fruits await them every day. But there is a reverse to this picture. In the dry season on the plains, the acacias cease to grow. No young leaves are produced, and the old glands do not secrete honey. Then want and hunger overtake the ants that have revelled in the luxury all the wet season; many of the thorns are depopulated, and only a few ants live through the season of scarcity. As soon, however, as the first rains set in, the trees throw out numerous vigorous shoots, and the ants multiply again with astonishing rapidity*." (Belt 1874 [1985], p. 221-2)

Based on these observations, it appears that Belt recognized that the following three factors were necessary to understand this apparently mutualistic interaction: (1) variation in herbivory risk, (2) variation in plant's abiotic conditions, and (3) variation in bodyguard defense. Yet despite his early recognition of these factors, most modern researchers have failed to appreciate their critical role in explaining the ecology and evolution of bodyguard food rewards.

Although largely ignored, a few biologists have made important contributions to our understanding of the variation in plant-bodyguard relationship (Bronstein 1994). In particular, Keeler (1981b) developed a concise model for understanding the evolution of the extrafloral nectary-ant guard mutualism. This model captures the fundamental conditions necessary for explaining why a plant may or may not invest in extrafloral nectar or nectaries, and based on it, Keeler made several specific, testable predictions about where nectaries should be favored and their resulting distributions. Similarly, Cushman and others (Cushman and Whitham 1989, Cushman 1991, Cushman and Addicott 1991) have lucidly argued that the outcome of homopteran-ant associations are environmentally "conditional" (i.e., the results of the homopteran-ant interactions strongly depend on the ecological settings in which they occur). Although extrafloral nectar and honeydew secretions from Homoptera are functionally similar (Koptur 1992a), the insights from research on homopteran-ant interactions have not been clearly extended to plants with extrafloral nectar. Most investigations of plant bodyguard rewards have continued to view these traits as static life history attributes, focusing instead on demonstrating whether or not a particular system is mutualistic (or at least beneficial to the plant), not on how and why the occurrence and strength of the interaction varies in space and time (Cushman and Addicott 1991, Bronstein 1994).

The objective of this paper is to organize these prior observations into a general framework for understanding the evolution of plant food rewards and their phenotypic variation. My goal is to provide a *raison d'être* ("reason for being") for plant food rewards, as Fraenkel did for plant secondary compounds (Fraenkel 1959), with the hope that it will further inquiry into this interesting but little studied topic. Some of what I present here is new, but the framework is founded on the ideas presented in the above papers, to which I hope to draw further attention. I focus in this paper on plant nectar rewards (i.e., secretions from extrafloral nectaries, or EFNs), but the proposed framework may be extended to other kinds of bodyguard rewards as well (e.g., food bodies and shelter). In the first part, I review what is known about variation in extrafloral nectaries and bodyguard defense. I then present the proposed framework, and explain how it is useful for understanding EFN plants. And finally, I suggest several hypotheses about the behavior and distribution of EFNs and discuss the evolutionary and ecological implications of such conditionality. I conclude by highlighting questions for future research.

2. EXTRAFLORAL NECTARIES

An extrafloral nectary is defined as any nectar secreting gland located outside flowers and not involved in pollination (Bentley 1977a, Koptur 1992a). They are extremely common, occurring in at least 93 plant families and 332 genera (Koptur 1992a). In certain habitats, plants with EFNs may be particularly frequent; for example, in the cerrado woodlands of Brazil, as many as 25% of woody plant species may bear EFNs, accounting for 31% of woody individuals in a given area (Oliveira and Leitão-Filho 1987, Oliveira and Oliveira-Filho 1991). Nectar secreting glands are also found on non-flowering plants (e.g., the leaves of some ferns), in which case the glands are correctly called "folial nectaries," or simply "nectaries" (Koptur 1992a). In this paper, however, I generically refer to all such glands as "extrafloral nectaries" (or EFNs) because the evolutionary forces that shape their evolution are likely to be similar. Given their prevalence, EFNs may be one of the most common types of bodyguard rewards.

Although EFNs are borne outside the flower, their position on plants is quite diverse, with EFNs sometimes being found on leaf blades (upper and lower surfaces), leaf margins, petioles, stipules, stems, bracts, pedicels, and even cotyledons (Elias 1983, Koptur 1992a). The aqueous secretions from EFNs have been documented to contain a variety of sugars (primarily sucrose, glucose, and fructose), amino acids, and other organic compounds (Bentley 1977a, Baker et al. 1978, Koptur 1979, Inouye and Inouye 1980, Ruffner and Clark 1986, Smith et al. 1990, Galetto and Bernardello 1992, Koptur 1994, Heil et al. 2000). In dry environments, the water content itself may also be an important bodyguard reward (Ruffner and Clark 1986).

2.1. EFN function

Despite Thomas Belt's early observations, the function of EFNs has been debated among biologists (reviewed by Bentley 1977a, Beattie 1985, Koptur 1992a). For example, some biologists have argued that EFNs serve as release valves for excess carbohydrate production. But in many systems, it has been shown that EFNs attract arthropod predators and parasitoids, which then serve as plant bodyguards against attacking herbivores (Bentley 1977a, Buckley 1982, Koptur 1992a). The loss of plant tissue by consumers is an important selective force in the evolution of plant phenotypes (Marquis 1992, Abrahamson and Weis 1997). In particular, it has been suggested that many plant secondary metabolities have evolved in response to selection by herbivores (Fraenkel 1959, Ehrlich and Raven 1964). For these chemical defenses, ecologists have proposed several hypotheses that make predictions about the amounts and kinds of defenses in plants (e.g., Feeny 1976, Rhoades and Cates 1976, Bryant et al. 1983, Coley et al. 1985, Herms and Mattson 1992; reviewed by Hartley and Jones 1997). Selection pressure by herbivores may have also resulted in the evolution of plant traits that encourage predation or parasitism of herbivores (Marquis and Whelan 1996, Agrawal 2000). Thus, the bodyguard hypothesis is at present the most supported explanation for the function of EFNs, although in the case of carnivorous plants, EFNs may also function to attract arthropod prey (Juniper et al. 1989).

Because these defenses act through a third party (e.g., ants and wasps), they have been referred to variously as indirect, extrinsic, or biotic defenses (Agrawal 1998, Heil et al. 2001a), although in this paper, I use the phrase bodyguard-mediated defense, or simply bodyguard defense. These defenses are also often referred to as mutualisms because both the plant and bodyguards are assumed to benefit from the interaction, but in most situations mutual benefit has not been demonstrated (Cushman and Beattie 1991). Therefore I avoid use of this term.

2.2. Why does bodyguard defense vary among plants?

Although EFNs have been documented to attract plant bodyguards, there are several cases in which EFNs could not be shown to provide any plant benefit (Janzen 1975, O'Dowd and Catchpole 1983, Tempel 1983, Lawton and Heads 1984, Whalen and Mackay 1988, Rashbrook et al. 1992, Gaume and McKey 1999, Zachariades and Midgley 1999). Based on these observations, there is a growing awareness that EFN bearing plants often vary in the level of defense by bodyguards. Why?

Three primary hypotheses are proposed to explain this variation in bodyguard defense: (1) bodyguard defense hypothesis, (2) herbivory risk hypothesis, and (3) resource limitation hypothesis. The relevance of the latter hypothesis is the least appreciated, and therefore the least studied. For this reason, I discuss this hypothesis below to a greater extent than the others.

2.2.1. Bodyguard defense hypothesis

Plants will not benefit from their EFNs if bodyguards are absent or ineffective (Bentley 1976, 1977a, Keeler 1979, 1981b, Boecklen 1984, Horvitz and Schemske 1984, Koptur 1985, Barton 1986, Smiley 1986, Whalen and Mackay 1988, Mackay and Whalen 1991, Koptur 1992b, Rashbrook et al. 1992, Zachariades and Midgley 1999). Bodyguard effectiveness may depend upon the capacity of herbivores to resist attack (Koptur 1984, Heads and Lawton 1985, Freitas and Oliveira 1996), and also upon the abundance and identity of the bodyguards, among other reasons (Table 4.1, Table 4.2). Because of their conspicuousness, the bodyguard role of ant visitors to EFNs has received by far the most attention (Bronstein 1998). However, a wide variety of non-ant arthropods are also often observed at EFNs (Keeler 1978, Smiley 1978, Price et al. 1980, Stephenson 1982, Tempel 1983, Lawton and Heads 1984, Hespenheide 1985, Koptur 1985, Treacy et al. 1987, Koptur and Lawton 1988, Koptur 1991, 1992a, Pemberton and Lee 1996, de la Fuente and Marquis 1999, Ruhren and Handel 1999, Zachariades and Midgley 1999), and some of these other plant visitors may also serve as plant bodyguards (e.g., wasps and spiders). By focusing on ants, many prior studies of EFNs may have missed the important contribution of these non-ant species. But if effective bodyguards are absent, for whatever reason, EFNs will not be favored by natural selection, and may be lost (Keeler 1985).

2.2.2. Herbivory risk hypothesis

Similarly, if herbivore impact is low or absent, plants will experience little benefit regardless of how many or what kinds of bodyguards are present (Keeler 1981a, Barton 1986).

But what determines herbivory risk to a plant? Numerous factors, including availability of herbivores, effectiveness of alternative chemical and physical defenses, and the plant's tolerance of herbivory (Herms and Mattson 1992, Marquis 1992). Therefore, if the risk of herbivory is negligible, either because herbivores are absent or the plant is already protected by alternative defenses, EFNs are unlikely to be favored by natural selection (Keeler 1981b, Barton 1986).

2.2.3. Resource limitation hypothesis

If plant resources are limited or needed for alternative physiological demands, the allocation of resources (e.g., carbon and nitrogen) to extrafloral nectar may be insufficient to attract plant bodyguards, and therefore the plant's EFNs will be non-functional. The critical resources of plants fall naturally into three categories: water, carbon, and nutrients (Bloom et al. 1985). The relative supplies of these critical resources differ greatly among habitats and temporally (Bloom et al. 1985). For example, in forested communities, light is perhaps the most spatially and temporally heterogeneous environmental factor facing plants (Pearcy 1999), but in other environments, water or soil nutrients may be more heterogeneous and limiting.

Many studies have shown that light and soil nutrient availability can alter plant abiotic defenses (e.g., chemical and physical defenses; Bryant et al. 1983, Herms and Mattson 1992, Marquis 1992, Gershenzon 1994); thus, it is likely that heterogeneity in the abiotic environment also affects EFN production or composition. This hypothesis posits that resource availability influences bodyguard defense by altering the amount and composition of EFN. If variation in the abiotic environment causes differences in extrafloral nectar production, it is likely that bodyguards will respond to this variation among plants, resulting in differences in herbivory and ultimately in plant fitness.

Most theoretical explanations for resource allocation to plant defenses assume that trade-offs exist between defense and other plant requirements (e.g., growth and reproduction; Bryant et al. 1983, Herms and Mattson 1992, Baldwin et al. 1998). In a similar fashion, trade-offs may exist for the allocation to bodyguard food rewards, such that resource allocation to bodyguard defense may be costly (in terms of fitness) if growth and reproduction is reduced under conditions not requiring defense (i.e., where herbivores are absent). Therefore, if there are fitness costs to nectar production (Pleasants and Chaplin 1983, Southwick 1984, Pyke 1991), plants growing in different habitats will face different fitness trade-offs associated with their phenotypic plasticity of resource allocation, and natural selection should favor plants that produce levels of extrafloral nectar appropriate to the herbivory risk and bodyguard defense. If phenotypic plasticity in extrafloral nectar production is genetically based, then plants that minimize the cost of these trade-offs in a particular environment, while maximizing the benefit, will be favored by natural selection.

Very little is known about the effect of the abiotic environment on bodyguard food rewards (Bentley 1976, Folgarait and Davidson 1994, 1995, Heil et al. 2001a). However, *floral* nectar production and chemical composition are well-known to be strongly influenced by light intensity, soil water availability, and other environmental conditions (see below), and therefore extrafloral nectar or nectaries are predicted to be similarly influenced by abiotic conditions. Also, the consequences of variation in extrafloral nectar production are analogous to those resulting from variation in floral nectar, where nectar availability is closely linked to reproductive success (Boose 1997). Similarly, there is growing evidence that honeydew secretions from homopterans is strongly influenced by the plant's environment (Cushman and Whitham 1989, Cushman 1991, Cushman and Addicott 1991), which suggests that secretions from EFNs may also show environmental conditionality.

2.2.4. Other hypotheses

Other hypotheses may also explain why EFNs appear to be non-functional. First, EFNs may represent "ghosts" of mutualism past (*sensu* Connell 1980). If this is the case, EFNs may have evolved to attract bodyguards in the past, but they are non-functional at present because herbivory risk or bodyguard defense has declined. However, for non-functional EFNs to persist in a population, two explanations are possible: (1) genotypic variation for EFN expression is completely absent from the plant populations, such that it is impossible for natural selection to favor nectary-less genotypes, or (2) the fitness costs of nectar production are very low, such that the loss of the EFNs is not favored by selection. Given enough time, however, it seems likely that genetic variation would arise, such that non-functional EFNs would be eliminated, even if they have low fitness costs.

Second, EFNs may serve a function other than bodyguard defense, such as excretion of excess photosynthates, deterrence of flower plunderers, or even to attract ant seed dispersers (O'Dowd and Catchpole 1983, Beattie 1985, Koptur 1992a, Zachariades and Midgley 1999, Wagner and Kay 2002). These hypotheses may explain certain situations, but they are probably not relevant to most EFN plants.

3. THE GENERAL FRAMEWORK

The original insight made by Keeler (1981b) is that these three hypotheses can be combined to predict the evolution of the extrafloral nectary-ant guard mutualism, i.e., that such mutualisms will be favored when (1) ants are present and effective bodyguards, (2) herbivore damage is high, and (3) the fitness cost of investment in nectar or nectaries is low. Keeler's (1985) model also includes the effectiveness of alternative defenses against herbivores (e.g., chemical defenses), but for simplicity, I have incorporated this variable into the herbivory risk component. A variety of hypotheses have been proposed to explain why plants may differ in the amounts and kinds of defenses (reviewed by Hartley and Jones 1997), but for my purposes here, it is not necessary to consider the particular nature of the relationship between bodyguard protection and other defenses.

Therefore, I have simplified Keeler's model into a graphic framework defined by just three axes of variation: (1) availability of excess resources for nectar allocation, (2) risk of plant damage by herbivores susceptible to plant bodyguards, and (3) effectiveness of visitors to EFNs as plant bodyguards (Fig. 4.1). The framework is equally applicable to all kinds of potential bodyguards (e.g., ants, wasps, and spiders) and does not depend on whether the bodyguard participant is in fact benefiting from the interaction. Thus, based on this framework, EFNs are predicted to evolve in plant populations where the favored states overlap (defined by the three axes) and to be selected against when resource availability, herbivory risk, or bodyguard defense is low (Fig. 4.2).

Besides its predictions about evolutionary changes, this general framework is also useful for explaining ecological variation in bodyguard defense. If plant populations growing in different environments experience different levels of heterogeneity in each of these three axes, natural selection may favor the evolution of phenotypic plasticity in nectar production or nectary expression (Fig. 4.3; Lloyd 1984). For example, in highly heterogeneous light environments, the framework predicts that plants that produce nectar only when excess resources are available would be favored over plants that produce nectar at either constant low or high levels. Thus, the framework predicts that plants growing in heterogeneous environments, whether due to variable resource availability, herbivory risk, or bodyguard effectiveness, should show greater plasticity of nectaries and nectar production than plants growing in more uniform environments (Fig. 4.3). Plants with plastic traits may have greater fitness than plants able to respond in only one way, but it is important to recognize that EFNs may be environmentally plastic without the plasticity being adaptive (Ackerly et al. 2000).

So far, I have illustrated the selection space defined by these three axes of variation as a simple cube containing eight alternative states (Fig. 4.1-4.3). This is not meant to imply that EFNs will be favored in a simple, yes/no fashion; the figure is intended merely to simplify the illustration. Additional insights may be gained by extending the framework to more realistic cost-benefit functions (Fig. 4.4). For example, if fitness costs of extrafloral nectar production are very low (e.g., in a high light environment), then EFNs are expected to be beneficial across a wider range of herbivore risks than if fitness costs are high (Fig. 4.4b). Similarly, if the costs of EFN are very low, EFNs are expected to be beneficial across a wider range of bodyguard availabilities (Fig. 4.4c).

3.1. Scale of EFN variation

Variation in extrafloral nectar production may occur at three scales: (1) variable number of modules (e.g., leaves) per plant, (2) variable number of nectaries per module, and (3) variable nectar production per nectary (quantity and quality). Knowledge of the scale of variation is important because it may influence bodyguard visitation. For example, egg parasitoid wasps may be unable to detect differences in nectar production among plants in different light environments if variation only occurs at the whole-plant scale because these tiny wasps visit few nectaries per plant and therefore would not detect differences among plants. In contrast, larger mobile predators (e.g., ants and vespid wasps) may respond to all scales of plasticity because they feed from many EFNs and modules per plant.

3.2. Why might genotypic variation in EFNs be expected?

Natural plant populations often contain genetic variation for traits associated with resistance to herbivores (e.g., Elle and Hare 2000); thus, it is likely that EFNs are also variable, especially considering that the effectiveness of EFNs depends on the presence of both herbivores and natural enemies. One possible reason for the maintenance of genetic variation for resistance traits is that they are costly (i.e., they lower fitness) in the absence of herbivory or when the resources required for the trait are in short supply (Herms and Mattson 1992, Simms 1992, Bergelson and Purrington 1996). Thus, genetic variation for resistance traits is expected to be found if variation in herbivory risk, bodyguard defense, or availability of limiting resources exists among environments (Elle and Hare 2000).

4. FLORAL VS. EXTRAFLORAL NECTAR

Very little in known about how environmental variation influences EFNs (see discussion below). In comparison, much more is known about environmental effects on homopteran honeydew-secretions (Cushman and Whitham 1989, Cushman 1991, Cushman and Addicott 1991, Breton and Addicott 1992b) and on floral nectar. Although some homopterans can be considered to be EFN analogs, floral nectaries are better studied and may be more comparable to EFNs because the interaction is not mediated by an intervening organism. Here I briefly review what is known about variation in floral and extrafloral nectar.

4.1. What is known about the variability of floral and extrafloral nectar?

Extrafloral nectar and nectaries can vary in many ways, including nectar quantity, composition, and timing, as well as in position and morphology (Table 4.3). For floral nectar, some of this variation has been attributed to genetic differences (Mitchell and Shaw 1993, Boose 1997, Klinkhamer and van der Veen-van Wijk 1999). Genotypic variation has also been found for extrafloral nectar or nectaries (Table 4.3), but unlike for floral nectar, too few studies have been completed to reveal general patterns that might be related to bodyguard visitation. Nevertheless, correlations have been found between the chemical contents of extrafloral nectar and nutritional requirements of nectary visitors (Baker et al. 1978, Lanza et al. 1993, Koptur 1994), and between the composition of plant food bodies and their consumers (Heil et al. 1998).

Field studies frequently find considerable variation in the quantity and composition of floral nectar, both among (Baker and Baker 1983) and within species (Pleasants and Chaplin 1983, Hodges 1993, Lanza et al. 1995, Boose 1997), and among flowers within individual plants (Devlin et al. 1987). Usually this observed phenotypic variation is attributed to environmental noise, but some studies have attempted to determine what specific environmental conditions are responsible (reviewed by Rathcke 1992, Table 4.4). Extrafloral nectar appears to be similarly influenced by abiotic environmental conditions, bodyguard feeding, and herbivory (Table 4.4), but again much less is known, and most of the observations have not been made under controlled experimental conditions. Several studies have shown that extrafloral secretion or composition can change in response to herbivory (Table 4.4), indicating that extrafloral nectar may function at times as an inducible defense (Koptur 1989, Agrawal and Rutter 1998).

For both flowers and EFNs, it is known that the accumulation of nectar can suppress further nectar secretion (Table 4.4). For example, in *Macaranga tanarius* (Euphorbiaceae), EFN secretion decreases when nectar is not removed by insects, and repeated removal of EFN during a 24 h period resulted in higher nectar production per day than a single nectar removal at the end of this period (Heil et al. 2000). Similar patterns have been observed for floral nectar (Pyke 1991). Thus, allocation of resources to nectar may depend on the availability and frequency of arthropod visitors. Some plants are also known to reabsorb floral nectar not collected by pollinators, which allows these plants to recover some of the energy cost, but this is unknown for EFNs. Nectar production would be considered costly if its production results in a reduction in other plant functions. In some situations, floral nectar production has been found to be costly (Pleasants and Chaplin 1983, Southwick 1984, Pyke 1991), whereas in others it has not (Bazzaz et al. 1979, Harder and Barrett 1992). In drought stressed environments, floral nectar production may be costly because of water loss (Nobel 1977, 1987, Whiley et al. 1988, Galen et al. 1999, Galen 2000). Reabsorption of nectar may be favored in plants with short-lived flowers (Nepi et al. 2001); thus, reabsorption of EFN may be unlikely because it is presented at the nectary for a more indefinite period (Koptur 1992a).

4.2. What is known about the effects of floral and extrafloral nectar variability?

Plants produce floral nectar to attract animal pollinators (Cruden et al. 1983, Bell 1985), and there is strong evidence that EFN plants produce nectar to attract bodyguards (see above). Therefore, if nectar production is environmentally influenced, the abiotic environment may alter plant attractiveness to pollinators and bodyguards. Clearly this is the case for pollinators. For example, pollinators alter their foraging behavior in response to variation in nectar levels, and these changes in behavior can alter plant fitness (Mitchell 1993, Dreisig 1995; reviewed by Rathcke 1992). Therefore, flowers that produce more nectar receive more pollinator visits, both among species (Southwick et al. 1981, Lack 1982) and within species (Thomson et al. 1982, Galen and Plowright 1985, Cresswell 1990, Mitchell 1993, Hodges 1995, Strauss et al. 1996, Lehtilä and Strauss 1997, Williams 1997, Mitchell et al. 1998, Carroll et al. 2001). Since plants with high nectar production receive and disperse more pollen, natural selection may lead to higher nectar production rates, such that plants may actively increase pollinator visitation by increasing flower rewards (Ladio and Aizen 1999).

Similarly, ant bodyguards appear to respond to abiotic variation in nectar availability (Bentley 1977b, Wirth and Leal 2001), although few studies have explicitly considered ant recruitment and defense in response to environmentally induced variation in quality or quantity of extrafloral nectar (de la Fuente and Marquis 1999). If nectar production by EFNs varies with biotic (herbivory) and abiotic stress, patterns of bodyguard visitation be influenced more by environmental conditions than by genotypic differences among plants. Numerous studies have shown that ants respond to differences in sugar baits, including experiments using simulated *Passiflora* nectar (Lanza and Krauss 1984, Lanza 1988, 1991, Lanza et al. 1993), resulting in different ant visitors. Also, ants are known to select aphid populations that produce large amounts of honeydew, responding to increased droplet size, renewal rate, population size, and density (Addicott 1978, Breton and Addicott 1992a, Mailleux et al. 2000).

5. PREDICTIONS

In the previous sections, I have discussed the proposed general framework for understanding the evolution and variation in EFNs. Here, in the last section, I consider several ecological and evolutionary predictions that are derived from the proposed framework, or at least are consistent with it. These predictions suggest answers to the following questions. Why do some species have EFNs and others do not? Why do some species have more or fewer EFNs than other species? Why do some species produce more or less extrafloral nectar, or nectar of different compositions? Why do different species have EFNs in different positions? Why do some habitats contain more or fewer plant species with EFNs? Why do some plants have more bodyguards or have better bodyguard defense than other plants? Why do some individual plants or plant parts produce different numbers of EFNs or nectar of different quality or quantity? Why are some EFN plants attacked more heavily by herbivores than other plants? Why are EFNs functional on some plants and not on others? And these are just some of the questions addressed by the proposed framework!

5.1. Ecological predictions

5.1.1. Environmental conditions will alter nectar quantity and quality

This prediction is strongly supported, based on observations from floral and extrafloral nectar (Table 4.4), although it needs to be experimentally tested to determine the particular relationship between plant resources and nectar production. Light availability in particular should have strong effects on EFN because it is predominantly carbon-based (Bentley 1977a, Koptur 1992a). Thus, in carbon-limited environments (i.e., in the shade), the costs of bodyguard defense may exceed the benefits, and therefore, other influences being equal, shaded plants should be expected to produce less nectar than plants in full sun. Nutrient limitation, however, should have less of an effect on nectar production, unless allocation to nutrient-based defenses is influenced by the relative availability of carbon and nutrients, as predicted by the Carbon/Nutrient Balance hypothesis (Bryant et al. 1983, Herms and Mattson 1992). For example, carbon may be available for allocation to nectar only when nitrogen is limiting, because when nitrogen is not limiting, most carbon is allocated to plant growth to avoid compromising competitive ability. Based on this hypothesis, it can therefore be predicted that nectar production by nitrogen-fixing plants, which frequently have EFNs (Koptur 1992a), will be relatively insensitive to variation in soil nitrogen availability, but will be highly sensitive to slight reductions in light availability.

5.1.2. Environmental conditions will alter bodyguard defense

Environmental conditionality of nectar production should also lead to nectar-mediated changes in the identity, abundance, or aggressiveness of plant bodyguards. For example, shaded plants may simply not be visited by potential bodyguards, and therefore be left unprotected. Davidson (1997) has suggested that the carbohydrate/protein ratios of extrafloral nectar may control the identities of ant associates and the quality of bodyguard protection, such that aggressive ants (strong competitors) on sun plants with high nectar production may be able to displace more timid ants (poor competitors), thereby restricting the timid ants to slow-growing shade plants (Davidson et al. 1991). Whether this has any consequence to plant protection is unclear because timid ants are sometimes good plant bodyguards (Table 4.1).

Alternatively, Palmer *et al*. (Palmer et al. 2000) suggested that the identity of ant bodyguards may be related to the availability of off-plant resources that are positively correlated with host plant growth rate, not specifically by variation in plant food rewards. For example, fast-growing plants may be occupied by superior ant competitors because these plants tend to grow in sites richer in protein food resources (e.g., invertebrate prey) only indirectly related to the availability of plant-derived resources (e.g., extrafloral nectar).

5.1.3. Environmental conditions should alter the decline in EFN production with leaf age

A decline in EFN production has been observed on several plant species (Koptur 1992a, de la Fuente and Marquis 1999). This pattern is consistent with the observation that young developing leaves are most at risk of herbivory (Coley and Aide 1991, Coley and Barone 1996) and have the highest potential value to the plant (McKey 1974, Rhoades 1979). But others have observed little change in nectar production with increasing leaf age (Stephenson 1982, Wickers

1993, 1997, Gaume and McKey 1998). I suggest that these conflicting observations may be explained in part by the resource limitation hypothesis above. If nectar production is influenced by environmental conditions, as predicted above, the decline in production with leaf age should vary among environments, such that the leaves of plants growing in resource-poor environments will employ bodyguards for a shorter duration than leaves on plants growing in resource-rich environments (Fig. 4.5).

5.1.4. Nectar production and bodyguard defense should vary among plant parts

Investigators have occasionally observed a correlation between ant foraging and the spatial production of extrafloral nectar within the foliage of individual plants (O'Dowd 1979, Beckmann and Stucky 1981, Koptur 1984, McKey 1984, Oliveira 1997), typically with greater ant visitation on younger leaves. McKey (1984) suggested that the distribution of ants among different plant parts is related to the tissue's vulnerability to herbivores, such that nectar is allocated only to tissues that actually require ant protection. Therefore, poorly defended young leaves should be well patrolled by ants, and well-defended older leaves should be little patrolled by ants. This hypothesis is entirely consistent with the proposed general framework for the evolution of EFNs, although the observed foraging pattern of bodyguards is also expected to be related to the module's (e.g., the leaf's) exposure to light and to accessibility of the plant part to bodyguards.

5.1.5. Nectar production and bodyguard defense should decline during succession

During the life cycle of pioneer plants, light levels are initially high and then gradually decline as the canopy gap closes. In such situations, bodyguard defenses are expected to be high initially, and then to decline as the gap closes, paralleling the observed decline in height growth rate (Ackerly 1996).

5.1.6. Diurnal and seasonal variation in EFN function should be expected

Diurnal and seasonal variation in extrafloral nectar secretion has been documented for several species (Table 4.3). Considering only the availability of photosynthates, nectar secretion should be generally found to be greater during the daylight hours, and during the sunnier periods of the year, because this is when bodyguard defense is expected to be cheapest. But for some species, ant visitation has been found to be greater at night (Fisher 1992, Gaume and McKey 1999, Oliveira et al. 1999). Based on the proposed framework, such disjuncts might be expected if herbivory risk was greater at night, therefore favoring the storage of photosynthates for later conversion to nectar, or possibly ants avoid foraging during the day because of higher predation/parasitism risk or avoidance of inhospitable environmental conditions.

5.1.7. Community-level consequences

Smiley (Smiley 1978) found that *Heliconius* egg parasitism was high in early-successional habitats (approaching 50%) but zero in the forest. The density of *Passiflora* vines (several species) was also higher in early-successional habitats than in the forest; thus, he suggested that the frequency of egg parasitism may depend on plant density. In contrast, he found that the frequency of *Passiflora* vines with foraging ants did not differ markedly between these habitats, suggesting that ant visitation is not related to habitat or plant density. I suggest an alternative explanation for these observations that could be of general relevance to plants with EFNs. If EFNs are much less productive on plants in forested habitats than on plants in the sun, the overall availability of extrafloral nectar may be much greater in sunny habitats (Schupp and Feener 1991, Feener and Schupp 1998). If this is the case, mobile natural enemies such as wasps may be attracted to sunny habitats, resulting in greater egg parasitism, and EFN plants in shaded habitat (forest) are well patrolled by ants (i.e., relatively immobile natural enemies) simply because the availability of extrafloral nectar in these habitats is a more limited resource.

5.2. Evolutionary predictions

The fitness cost of insect herbivory has repeatedly favored the evolution of plants with EFNs and other bodyguard rewards, to attract ants and wasps that prey upon or otherwise deter the herbivores (Price et al. 1980). But why don't more plant species have EFNs? The remainder of this section is directed at providing an answer to this question. Some of the predictions suggested here were also made by Keeler (Keeler 1981b). While reading the following predictions, it is important to keep in mind that gradients in bodyguard effectiveness, herbivory risk, and resource availability are often positively correlated, which will make these evolutionary predictions more difficult to test than the previous ecological ones.

5.2.1. EFN plants should be most common in resource-rich habitats

Because nectar is more costly to produce in light-limited environments (O'Dowd 1979), EFN plants should be most common in sunny habitats, where carbon is more likely to be available in excess of the requirements for growth and reproduction (Bryant et al. 1983, Herms and Mattson 1992), and fewest in shaded habitats. Similarly, the number of EFNs per leaf (or other module) should be greater in sunny habitats. In nutrient-rich environments, however, carbon may not be available in excess because it is allocated to growth. The cerrado vegetation of Brazil is an example of a habitat with extremely low fertility, and consistent with the prediction, the frequency of species with EFNs is very high (Oliveira and Leitão-Filho 1987, Oliveira and Oliveira-Filho 1991). In such habitats, the cost of extrafloral nectar production may be so low that even rare selection events (e.g., herbivore outbreaks) may be sufficient to maintain the expression of EFNs (Oliveira 1997).

Given the high fitness costs of water loss, desert habitats should have fewer plants with EFNs than do plants growing in environments with excess moisture availability. But EFNs have been observed on several species of *Cactus* (Pickett and Clark 1979, Ruffner and Clark 1986, Koptur 1992a, Rico-Gray et al. 1998, Oliveira et al. 1999). These plants are probably not limited by light availability; thus, the water content of nectar may be the most costly component. But if the habitats where these plants grow have limited soil nutrients, water too may be in excess supply (depending on rooting depth), which could explain how these plants can "afford" the nectar production.

In the wet tropics, plants with extrafloral nectaries are more frequent in light gaps and forest clearings (disturbed areas) than in the forest interior (Bentley 1976, Schupp and Feener 1991, Feener and Schupp 1998). In particular, plants classified as gap species are more likely to have EFNs (or to produce food bodies) than are plants classified as shade-tolerant species (Schupp and Feener 1991). One explanation for this observation is that effective ant defenders may be more abundant in areas of high light availability (Schupp and Feener 1991), but Feener and Schupp (1998) found that ant communities in gaps are not different from those in adjacent forest, suggesting that light availability is the primary determinant of the greater abundance of EFN plants in these habitats.

In my own research, I have noticed that *Passiflora* species with numerous EFNs tend to be those found most commonly in sunny habitats, whereas species with few or no EFNs appear to be restricted to heavily shaded areas.

5.2.2. EFN plants will be favored where herbivory is intense and disfavored where it is unimportant

Consistent with this generalization is the observation that EFN plants are more frequent in tropical than temperate habitats (Coley and Aide 1991, Koptur 1992a, Pemberton 1998), which is where herbivory risk is also greater (Coley and Barone 1996, Dyer and Coley 2002). Such patterns, however, may be a function of bodyguard effectiveness against different kinds of herbivores, not simply the overall level of herbivory. More detailed studies are therefore needed to test the generality of this prediction.

5.2.3. EFN plants will be favored where ants are omnipresent and disfavored where they are scarce

In habitats where bodyguards are absent, the cost of bodyguard rewards can be sufficient to favor the loss of these plant traits (Rickson 1977, Keeler 1979, 1985). For example, Keeler (1985) found that EFNs had been lost from plants growing in Hawaii where ants are absent, in comparison to mainland habitats where the plants are known to have EFNs and ants. It is unlikely that this observation could be explained by differences in resource availabilities between islands and the mainland, but it may be possible that herbivory risk as well as bodyguard effectiveness differ between these two locations. In light of the proposed framework, a further inquiry might compare sun vs. shade plants for the frequency of loss of EFNs. The framework would predict that the cost of non-functional EFNs is greater for shade plants, and therefore that the frequency (and speed) of loss would be greater for shade plants in these habitats without ants.

Gradients in ant abundance also occur between lowland and upland habitats (e.g., Olson 1994), and the decline in ant abundance with elevation may be responsible for a corresponding decline in the frequency of EFN plants (Keeler 1979, 1985). Similarly, I have noted in *Passiflora* that the number of EFNs per leaf also appears to decline with increasing elevation. For example, both petiolar and laminar EFNs are absent from the three highest ranging *Passiflora* species in Costa Rica (*P. apetala*, *P. membranacea*, and *P. sexflora*), which are restricted to elevations between 1100-2700 m, whereas EFNs are present on most *Passiflora* species found at low and mid-elevations. I suggest that natural selection has favored the loss of EFNs from these *Passiflora* species at high elevations because bodyguards (e.g., ants and wasps) are scarce in these habitats and because nectar is more expensive to produce there due to the limited light availability resulting from the persistent cloud cover (Cavelier 1996). In the Rocky Mountains, Inouye and Taylor (1979) found that ants were effective bodyguards of *Helianthella quinquenervis* (an EFN plant) at sites above 3000 m. This apparent greater effectiveness of bodyguard-mediated defenses in temperate mountains may be explained by the absence of a persistent cloud cover and the longer periods of warm, dry weather (Bruhl et al. 1999). To resolve the cause of EFN loss, therefore, further inquiry might compare the distribution of EFN plants on wet vs. dry tropical mountains.

5.2.4. Fewer cheaters on shade plants

As predicted above, EFNs should be costly to maintain on plants restricted to shaded habitats. Therefore, visitors to EFNs that provide no benefit to the host plant (i.e., nectar robbers) should be less common, or somehow prevented, on shaded plants than on plants in sunny habitats where nectar is cheap. Because extrafloral nectar is expected to be more costly to produce in the shade, plants with EFNs that grow in these habitats probably experience high herbivory, and therefore, natural selection must strongly favor EFNs despite their high cost. For this reason, it should also be expected that shade-restricted EFN plants would be more likely to evolve specialist relationships with "honest" bodyguards (see below).

5.2.5. Effectiveness of bodyguard defense should depend on plant life form

Extrafloral nectaries are found on all plant life forms, including herbs, shrubs, trees, vines, and epiphytes (Koptur 1992a), but some life forms may be more effective at attracting bodyguards, which may explain observed differences in nectary abundance or nectar secretion. For example, Bentley (1981) noticed that vines and lianas are often endowed with EFNs, and she argued that the climbing habit may encourage the evolution of EFNs because climbers have more plant-to-plant contacts and therefore greater access by foraging ants (also see Apple and Feener 2001). In contrast, the presence of plant-to-plant connections may be of little importance to wasp predators and parasitoids (or jumping spiders) because these flying bodyguards are not inhibited by the lack of physical connections.

The greater abundance of EFNs on vines and lianas might also be explained by the lower construction costs incurred by these plants compared to non-climbing taxa (Holbrook and Putz

1996); by taking advantage of neighboring vegetation for support, vines and lianas may be able to allocate more resources to bodyguard defenses, without incurring the costs of reduced growth rate and smaller leaf area. Similarly, pioneer trees (e.g., *Ochroma pyramidale*, balsa) with their very low wood density (King 1991) may have more carbon for allocation to bodyguard defenses than non-pioneer trees (O'Dowd 1979). Alternatively, bodyguard defenses may simply be more likely to evolve in gap species because they live in carbon-rich habitats, or possibly the observed pattern may be simply an artifact of phylogeny (Schupp and Feener 1991, Feener and Schupp 1998).

5.2.6. Plants with long-lived leaves should have fewer EFNs

The leaf life span hypothesis (McKey 1984, Fonseca 1994) predicts that bodyguard defenses should be restricted to plants with short life spans because the maintenance costs for these biotic defenses become prohibitively large relative to the declining photosynthetic value of the leaf tissue. On the other hand, plants with long-lived leaves should rely more on chemical and physical defenses because their initially high construction costs would be amortized over the life of the leaf (Fig. 4.6).

McKey (1984) proposed this hypothesis to explain why *Leonardoxa africana* (Caesalpiniaceae) uses chemical and physical defenses (i.e., tannins and lignins) to defend its very long-lived leaves (ca. 3 yrs) and bodyguard defenses (i.e., extrafloral nectar) to maintain a small ant force that protect young leaves only. Fonseca (1993), however, found that the Amazonian myrmecophytic canopy tree *Tachigali myrmecophila* (Caesalpinaceae) maintained very large ant colonies, yet produced leaves of extremely long life span (ca. 3.75 yrs; Fonseca 1994), which is inconsistent with the predictions of the leaf life span hypothesis.

I suggest that it may be possible to explain such contradictory results by incorporating the idea of environmental conditionality into McKey's (1984) hypothesis. If the cost of extrafloral nectar (or other food reward) is altered by the plant's abiotic environment, the cost of bodyguard defense may increase or greatly decrease, therefore either exaggerating or moderating the relative advantage of biotic vs. constitutive defenses (Fig. 4.6). For example, in high light environments, the cost of extrafloral nectar is expected to be much lower due to the greater availability of photosynthates (O'Dowd 1979), and therefore, in these environments, constitutive defenses will have little advantage over bodyguard defenses, regardless of leaf life span.

With this modification, the leaf life span hypothesis now predicts that long-lived leaves are most likely to be protected by constitutive defenses in light-limited environments (Fig. 4.6). But this does not help to explain the high level of ant defense in *T. myrmecophila* because it is shade tolerant and very slow-growing (Fonseca 1994). Therefore, as suggested by Fonseca (1994), the kind of defense strategy adopted by a plant may depend more on the specific benefit of the defense strategy employed, not simply on the costs of construction and maintenance. For example, *T. myrmecophila* appears to benefit a great deal from its high investment in ant defense (Fonseca 1994), which suggests defense by ants is worth the cost. Thus, the leaf life span hypothesis must also be modified to consider herbivory risk and reconsidered in light of idea general framework proposed here.

It is important to note that *L. Africana* and *T. myrmecophila* may not be strictly comparable, because the resident bodyguard ants in the latter system do not obtain food rewards directly from the plant, but rather indirectly, by harvesting honeydew from coccid colonies maintained inside the leaf domatia. Therefore, the allocation of plant resources to ant defense in *T. myrmecophila* may be higher than necessary, and greater than the leaf life span hypothesis would predict, because natural selection has been unable to favor more moderate levels of ant defense.

5.2.7. EFN position on the plant should vary with light, herbivores, and bodyguards

EFN position and morphology is highly variable among species (Table 4.3), and I suggest this variation may also be predicted by the proposed framework. In general, EFN position varies little among related species (Elias 1983, Schupp and Feener 1991), but in *Passiflora* it is highly variable. Depending on the species, EFNs in this genus are sometimes located on the abaxial surface of the leaf blade, on the leaf margin, on the petiole, in all three positions, and sometimes completely absent. Also, *Passiflora* EFNs seem to be most abundant on species growing in high light habitats, where they tend to be scattered across the leaf blade, and to be least abundant on species from low light habitats, where they tend to be restricted to the petiole (pers. obs.).

The specific location of EFNs on leaves is probably highly subject to natural selection because it determines the foraging movement of bodyguards. For example, O'Dowd (O'Dowd 1979) found that nectaries located at the distal ends of leaf veins in *Ochroma pyramidale* induced ant foraging across the entire leaf blade, which may therefore improve bodyguard defense.

In *Passiflora*, the group with which I am most familiar, the majority of species have EFNs located at the proximal end of the leaf blade or only on the leaf petiole. In shade tolerant species, the few nectaries present (usually 2) are usually only in these positions. This distribution and abundance of EFNs may be explained by the fitness value of these tissues (Rhoades and Cates 1976) because if these areas are damaged, the leaf will die (Zangerl and Bazzaz 1992). Therefore, I predict that EFNs are most likely to be located toward the distal end of the lamina on plants that grow in sunny habitats (e.g., *Ochroma pyramidale;* O'Dowd 1979), where carbon-based defenses are cheapest to produce, and to be located in these basal areas in shaded habitats.

Annual plants and perennial herbs tend to have nectaries near flowers and fruits (Koptur 1992a), which may also be related to the fitness benefits of protecting these structures.

5.2.8. EFNs should increase with plant size or age

In tropical forests, most plant species must be able to tolerate shaded conditions, at least during the early stages of growth (Clark and Clark 1992). Except for pioneer species, most plants will begin their lives in shaded microsites, and rarely will seedlings have surplus carbon that could be diverted to extrafloral nectar. Thus, the expression of EFNs and the production of extrafloral nectar should be unlikely for young EFN plants. The one observation of EFNs on cotyledons (cited in Koptur 1992a) is for *Ricinus communis* (castor oil plant, Euphorbiaceae), which tends to grow in high light environments.

5.2.9. EFN plasticity and environmental heterogeneity

As mentioned above, the availability of resources, herbivores, and bodyguards may influence EFN number, volume, etc. In heterogeneous environments, selection should favor genotypes with greater phenotypic plasticity than in relatively homogeneous environments (Sultan 1987, Lortie and Aarssen 1996, Balaguer et al. 2001). For example, over ecological and evolutionary time, plants growing in uniformly open habitat probably experience much less light heterogeneity than do plants growing in habitats with mixtures of light environments (e.g., forests with light gaps), and therefore these plants from open habitats may display less plasticity in EFN production than would plants from uniformly light or dark habitats (like Balaguer et al. 2001).

Because early and late successional tree species are thought to experience contrasting levels of light heterogeneity, early successional gap-dependent species may have greater physiological flexibility because they must cope with greater resource variability in environments (Bazzaz and Pickett 1980, Strauss-Debenedetti and Bazzaz 1996). Similarly, EFNs may be more plastic in gap species than in late successional species.

The degree of plasticity may also be influenced by plant lifespan. Long-lived organisms are expected to have greater developmental flexibility because they must face greater environmental variation during their life spans (Sultan 1987). Thus, longer-lived plants might be expected to display greater plasticity in their EFNs than short-lived plants (e.g., annuals).

The degree of plasticity may also be influenced by the plant's life form. Trees typically encounter a wide variety of light environments during their life span, as they grow from the forest floor into the canopy, whereas in contrast, the climbing habit of vines may enable them to avoid such variation (Holbrook and Putz 1996). Thus, vines may be better able to regulate their production of extrafloral nectar than trees during growth.

5.2.10. EFN plasticity and myrmecophytism

Environmental plasticity of food rewards should be lower for myrmecophytes because these plants clearly depend to a greater degree on their bodyguard defenses; ant-plant specificity is simply not expected to evolve in environments with variable cost/benefit ratios for EFNs (Rico-Gray 1993). Thus, the effects of the abiotic environment should be considerable for plants with facultative associations with ants, but negligible for plants with obligate associations (myrmecophytes). Moreover, selection may favor plants that can "sense" the presence of bodyguards and allocate extrafloral nectar only when bodyguards are available. One possible mechanism for such plasticity is the observed suppression of further nectar secretion in response to nectar accumulation (references in Table 4.4). Selection for such traits is expected to be strongest in habitats where plants benefit greatly from bodyguard defense, but where resources are scarce and bodyguards are often absent.

6. CONCLUSIONS

A major goal in the study of plant-herbivore interactions is to understand why plants differ in their commitment to defenses, within and among species, and hence in their susceptibility to herbivores. Since Thomas Belt's seminal observations of ants on acacia (Belt 1874 [1985]), numerous investigators have studied the bodyguard role of arthropod visitors at EFNs, focusing primarily on ants, yet surprisingly little attention has been given to the potential influence of biotic and abiotic environmental conditions on the functioning of the bodyguard defense. I have attempted in this paper to point out an interesting, but neglected, area of study that shows potentially fruitful directions for future research. Little detailed evidence presently exists for the evaluation of the hypotheses presented here, but I predict that much progress will be gained by pursuing the lines of research suggested here. In particular, future work should focus on how variation in the light and other environmental conditions causes changes in extrafloral nectar production, using experimental methods similar to those of Stephenson (1982). In addition, we need more descriptive studies of bodyguard defense across a variety of environmental conditions to improve our understanding of the plasticity of species interactions and outcomes. Finally, we need to study the effects of shading on extrafloral nectar production in nutrient-rich habitats, because based on the carbon-nutrient balance hypothesis (Bryant et al. 1983) variation in light availability is predicted to have little effect.

In this paper, I have proposed a general framework to explain the evolution and ecology of EFNs. Clearly it is highly speculative, and not all features of an organism are adaptations (Gould and Lewontin 1979), but I believe the evolutionary perspective taken here has proven useful to formulate interesting and testable hypotheses (Garland and Carter 1994, Rose and Lauder 1996). My goal has been to offer a general framework for thinking about EFNs and, thereby, to stimulate further inquiry into the causes of variation. Instead of ignoring it, researchers should seek out and embrace the natural variation they encounter in bodyguard interactions, within and among populations, as an important subject of study (Arntz and Delph 2001). Whether or not a system is mutualistic does not mean that other factors are not involved in a dynamic interaction. And regardless, much insight can be obtained by focusing on the selection pressures of a single participant.

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Table 4.1. Potential bodyguard variation among plants or plant parts. These bodyguard characteristics are analogous to variation in plant chemical defenses.

Potential variation in bodyguards	**References**
Identity	Bentley 1976, Schemske 1982, Horvitz and Schemske 1984, Koptur 1984, Rico-Gray and Thien 1989, Gaume and McKey 1999, Oliveira et al. 1999, Labeyrie et al. 2001
Abundance or density	Inouye and Taylor 1979, Thompson 1988
Aggressiveness	Janzen 1972, Bentley 1977b, Letourneau 1983, Horvitz and Schemske 1984, Oliveira et al. 1987, Gaume et al. 1997
Species richness	(hypothesized)
Movement among modules	(hypothesized)
Constancy of presence	Smiley 1986, Koptur and Lawton 1988
Host plant specialization	Fiala et al. 1994, Hossaert-McKey et al. 2001

Table 4.2. Potential environmental causes of variation in plant bodyguard defense. These potential causes of variation are classified as either extrinsic or intrinsic to the plant.

Potential cause of variation	**References**
Extrinsic:	
Availability of alternative food sources	Schupp and Feener 1991, Feener and Schupp 1998, Palmer et al. 2000
Nearness of plants to nest sites	Keeler 1978, Tilman 1978, Inouye and Taylor 1979, Barton 1986, Wagner 1997, Dejean et al. 2000
Temperature and humidity	Hölldobler and Wilson 1990
Intrinsic:	
Availability of domatia	Fiala et al. 1994, Blüthgen and Wesenberg 2001
Availability of extrafloral nectar	Bentley 1976, Koptur 1979, Boecklen 1984, Schupp and Feener 1991, Feener and Schupp 1998
Availability of food bodies	Heil et al. 1997, Heil et al. 2001a, Linsenmair et al. 2001

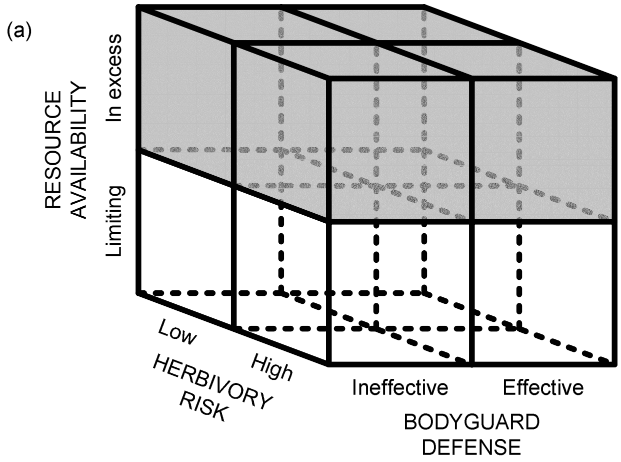
Table 4.3. Potential phenotypic and genotypic variation in extrafloral nectaries (EFNs) and their secretions. Some aspects of this variation may alter bodyguard visitation, or may serve to deter nectar robbers that provide no plant benefit.

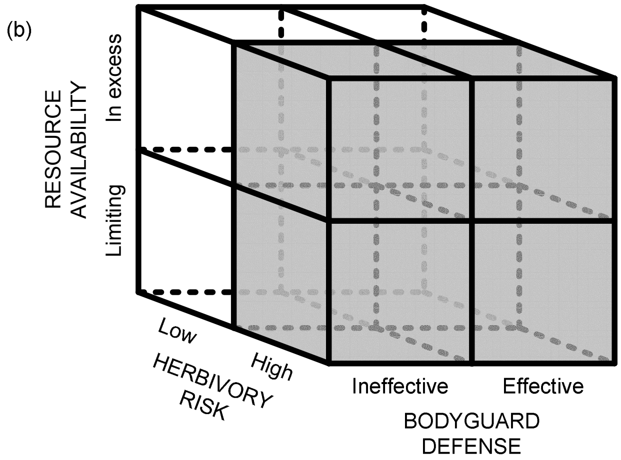
Potential variation	**References for floral nectar**	**References for extrafloral nectar**
Number (or density) of flowers/EFNs:		
per plant module (e.g., leaf)	(many studies)	Mackay and Whalen 1991
per plant	(many studies)	Young et al. 1997
Position	(many studies)	Elias 1983, Koptur 1992a
Size, shape, and pigmentation	(many studies)	Elias 1983, Koptur 1992a
Total nectar volume	(many studies)	Koptur 1994, Wäckers et al. 2001
Concentration	(many studies)	Wunnachit et al. 1992
Production rate of nectar (i.e., replacement rate)	(many studies)	
Composition of nectar:		
Sugars	(many studies)	Baker and Baker 1983, Wunnachit et al. 1992, Pascal 1993, Koptur 1994
Amino acids	Baker and Baker 1986, Rusterholz and Erhardt 1998, Gardener and Gillman 2001	Baker et al. 1978, Koptur 1979, Inouye and Inouye 1980, Ruffner and Clark 1986, Smith et al. 1990, Galetto and Bernardello 1992, Koptur 1994, Heil et al. 2000
Other substances	Adler 2000	
Timing of nectar production:		
Diurnal variation	Cruden et al. 1983, Pleasants 1983, Devlin et al. 1987	Bentley 1977b, Wickers 1993, 1997, Heil et al. 2000
Annual (seasonal) variation	(many studies)	Bentley 1977a, Tilman 1978, Rico-Gray 1993, Kawano et al. 1999
Module developmental stage (age)	Torres and Galetto 1998, Wesselingh and Arnold 2000	Bentley 1977b, Tilman 1978, O'Dowd 1979, Tempel 1983, Ruffner and Clark 1986, Heil et al. 2000
Plant age (size)	Pleasants 1983, Pleasants and Chaplin 1983, Devlin et al. 1987	Ruffner and Clark 1986

Table 4.4. Potential environmental causes of variation in floral and extrafloral nectar. Some of these factors will affect nectar directly (e.g., humidity, bodyguard visitation), whereas other factors influence nectar via a change in plant performance. If genotypic variation exists for the plant response to these causes, natural selection may favor the evolution of plant traits that minimize their costs.

Potential cause of variation	**References for floral nectar**	**References for extrafloral nectar**
Abiotic:		
Soil moisture	Cruden et al. 1983, Pleasants 1983, Zimmerman 1983, Zimmerman and Pyke 1988, Villarreal and Freeman 1990, Lee and Felker 1992, Wyatt et al. 1992, Campbell 1996, Boose 1997, Carroll et al. 2001	Beattie 1985, Ruffner and Clark 1986, Pascal 1993, Rico-Gray 1993, Wickers 1997, Wirth and Leal 2001
Light availability	Herrera 1995, Boose 1997, Aizen and Basilio 1998	Bentley 1976, de la Fuente and Marquis 1999
Soil nutrients	Gardener and Gillman 2001	Heil et al. 2001a, Linsenmair et al. 2001
Temperature and humidity	Cruden et al. 1983, Marden 1984, Freeman and Head 1990, Villarreal and Freeman 1990, Nicolson 1993, Jakobsen and Kristjansson 1994, Petanidou and Smets 1996	Koptur 1992a, 1994
Atmospheric CO_2 concentration	Osborne et al. 1997, Rusterholz and Erhardt 1998, Harrington et al. 1999, Lake and Hughes 1999, Dag and Eisikowitch 2000	(unknown)
Carbon/nutrient balance	(unknown)	(unknown, but see Davidson and Fisher 1991, Folgarait and Davidson 1994, 1995)
Biotic:		
Consumption by pollinators/bodyguards	(many studies)	(probably)
Consumption by nectar robbers	Maloof and Inouye 2000	(probably)
Nectar accumulation	Koptur 1983, Gill 1988, Pyke 1991, Vickery and Sutherland 1994, Torres and Galetto 1998, Wesselingh and Arnold 2000	Heil et al. 2000, Linsenmair et al. 2001
Nectar re-absorption	Búrquez and Corbet 1991, Nicolson 1995, Nepi et al. 1996, Koopowitz and Marchant 1998, Witt et al. 1999, Nepi et al. 2001	(unknown)
Herbivory	Hendrix 1988, Michaud 1989, Gottsberger et al. 1990, Michaud 1990, Marquis 1992, Strauss et al. 1996, Lehtilä and Strauss 1997, Strauss 1997, Ladio and Aizen 1999, Lehtilä and Strauss 1999, Cresswell et al. 2001, Strauss et al. 2001	Stephenson 1982, Koptur 1989, Smith et al. 1990, Agrawal and Rutter 1998, Wäckers and Wunderlin 1999, Heil et al. 2000, Heil et al. 2001b, Wäckers et al. 2001

Fig. 4.1. The proposed framework for the evolution of extrafloral nectaries (EFNs), based on selection gradients defined by three environmental factors: (a) resource availability, (b) herbivory risk, and (c) bodyguard defense. In general, EFNs are expected to be favored when plant resources for nectar are available in excess of other plant requirements [shaded region in (a)], when herbivory risks are high [shaded region in (b)], and when effective bodyguards are present [shaded region in (c)].


(a)
RESOURCE AVAILABILITY
In excess
Limiting
Low
High
HERBIVORY RISK
Ineffective
Effective
BODYGUARD DEFENSE


(b)
RESOURCE AVAILABILITY
In excess
Limiting
Low
High
HERBIVORY RISK
Ineffective
Effective
BODYGUARD DEFENSE

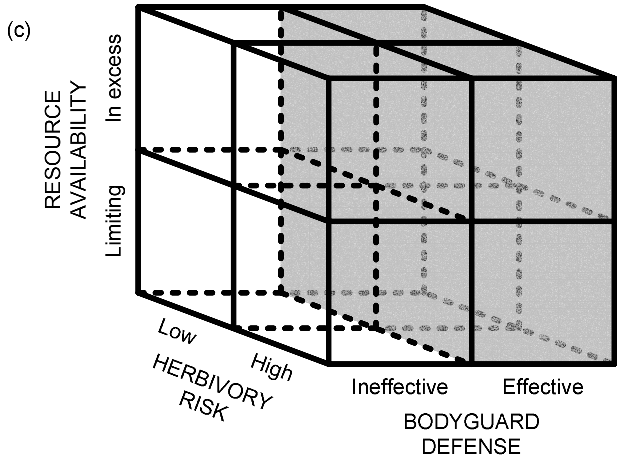

(c)
RESOURCE AVAILABILITY
In excess
Limiting
Low
High
HERBIVORY RISK
Ineffective
Effective
BODYGUARD DEFENSE

Fig. 4.2. The zone of strongest selection for EFNs (indicated by shading), defined by the union of the three selection gradients shown in Fig. 4.1.

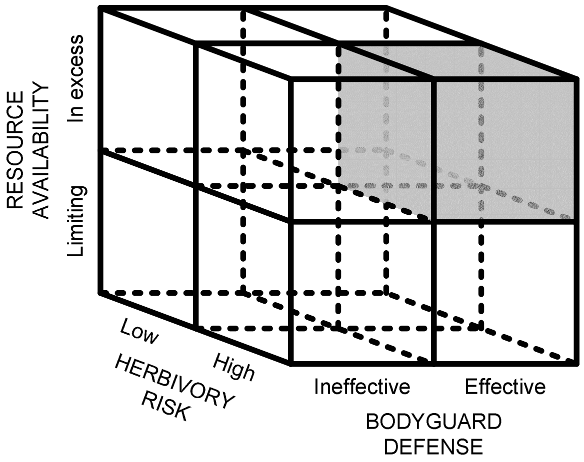

RESOURCE AVAILABILITY
In excess
Limiting
Low
High
HERBIVORY RISK
Ineffective
Effective
BODYGUARD DEFENSE

Fig. 4.3. Proposed framework for the evolution of phenotypic plasticity of extrafloral nectaries (e.g., variation in nectary number or nectar production). Two cells are shaded because heterogeneity in bodyguard defense is predicted to have little influence on EFN plasticity compared to heterogeneity in the resource environment or the risk of herbivory.

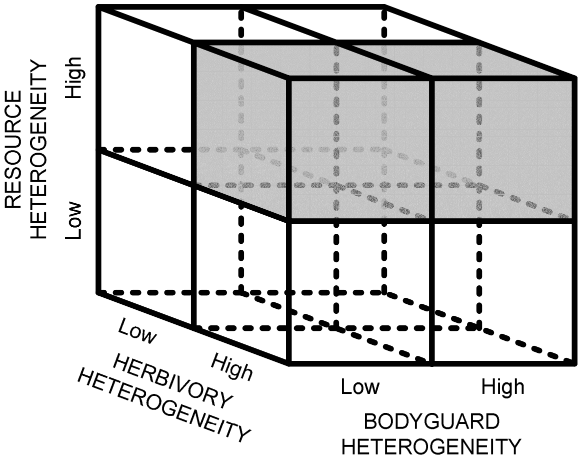

RESOURCE HETEROGENEITY
High
Low
Low
High
HERBIVORY HETEROGENEITY
Low
High
BODYGUARD HETEROGENEITY

Fig. 4.4. Several hypothetical cost-benefit scenarios for the evolution of EFNs. Each line represents the reaction norm of a genotype across an environmental gradient, partitioned into either fitness cost or benefit. Here, the cost-benefit functions are considered to be linear, for simplicity. The height of each line indicates the degree of fitness cost or benefit, and the slope represents the yield of each genotype in terms of fitness gains or losses. In (a), the fitness cost of EFNs is shown to decline with increasing resource availability. In this scenario, EFNs are predicted to be favored only when herbivory risk is high and effective bodyguards are present (BENEFIT #1, not BENEFIT #2), which is when fitness benefits exceed costs (shaded region).

In (b), the fitness cost of EFNs is shown to be independent of herbivory risk (slope = 0), but the fitness benefit of EFNs is predicted to increase with greater herbivory and bodyguards are present (BENEFIT #1). In this scenario, fitness benefits are shown to exceed costs across a wider range of herbivory risks when plant resources are more available (COST #2) than when they are scarce (COST #1).

In (c), the fitness cost of EFNs is shown to be independent of bodyguard defense (slope = 0), and the fitness benefit of EFNs is shown to increase with greater bodyguard defense, but only when herbivores are present (BENEFIT #1, not BENEFIT #2). Here, the fitness benefits of EFNs are predicted to exceed costs across a wider range of bodyguard defense when resources are more available (COST #2) than when resources are scarce (COST #1).

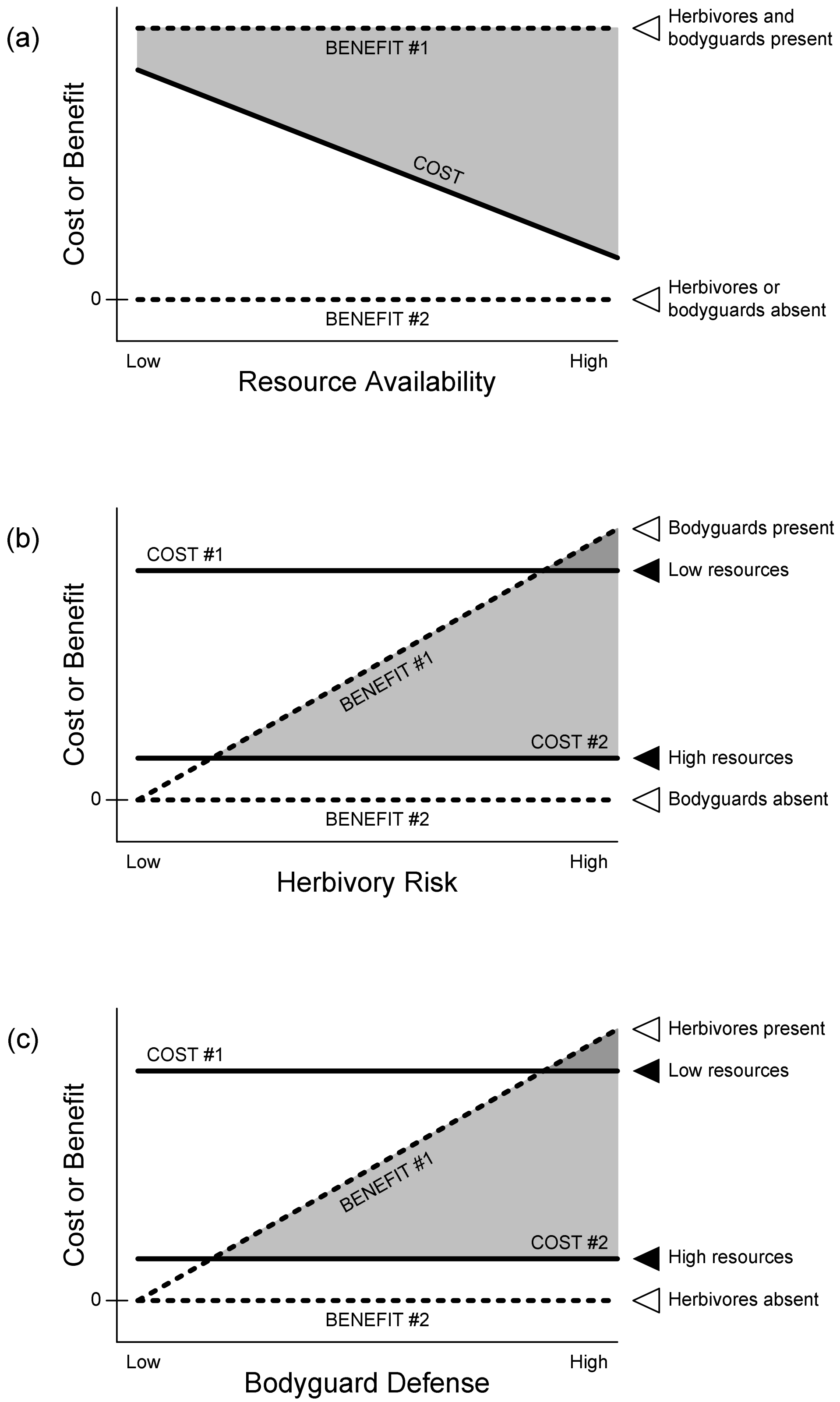
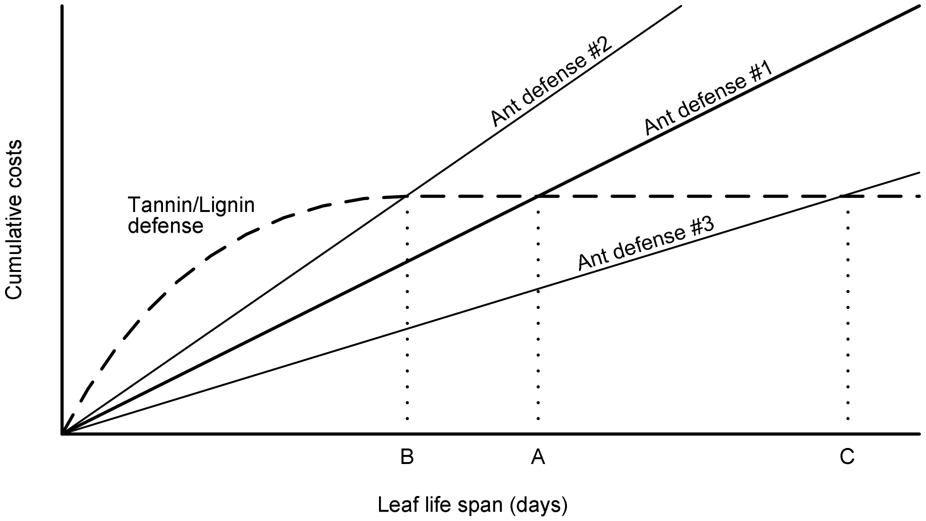

(a)
Cost or Benefit
BENEFIT #1
COST
0
BENEFIT #2
Herbivores and bodyguards present
Herbivores or bodyguards absent
Low
High
Resource Availability
(b)
Cost or Benefit
COST #1
BENEFIT #1
COST #2
0
BENEFIT #2
Bodyguards present
Low resources
High resources
Bodyguards absent
Low
High
Herbivory Risk
(c)
Cost or Benefit
COST #1
BENEFIT #1
COST #2
0
BENEFIT #2
Herbivores present
Low resources
High resources
Herbivores absent
Low
High
Bodyguard Defense

Fig. 4.5. Effect of the abiotic environment on nectar production and bodyguard defense. Leaves on plants growing in a resource-poor environment (solid line) are predicted to stop producing nectar at a younger age (point "A") than leaves on plants growing in a resource-rich environment (dashed line, point "B"). This effect of the abiotic environment is expected to have a corresponding effect on bodyguard abundance and plant defense.


Nectar production
A
B
Leaf age (days)

Fig. 4.6. McKey's (1984) leaf life span hypothesis, with a suggested modification. Tannins and lignins (dashed line) have high production costs, but once in place, they do not turn over and have no maintenance costs. In contrast, ant defense has low initial costs but high maintenance costs, such that eventually the cost of ant defense will exceed that of tannin/lignin defense. Point "A" indicates this turning point; leaves of shorter life span are predicted to be defended by ants, whereas leaves of longer life span are predicted to be defended by tannin/lignins. But if the cost of extrafloral nectar (or other food rewards) is altered by the plant's abiotic environment, the cost of ant defense may be either higher ("Ant defense #2") or much lower ("Ant defense #3"). Therefore, in circumstances where the cost of extrafloral nectar is very low, it might be expected that even leaves of long life span will be protected by ant defense (point "C"), as observed by Fonseca (1994).


Ant defense #2
Ant defense #1
Tannin/Lignin defense
Ant defense #3
Cumulative costs
B
A
C
Leaf life span (days)

CHAPTER 5

CONCLUSIONS

In my studies of *Passiflora biflora*, I have endeavored to develop a better understanding of the biotic and abiotic interactions affecting this Neotropical vine, or as Darwin might have put it, to untangle the entangled bank of this species. Contrary to my expectations, the results of my research demonstrate that *P. biflora* performance in sun and shade is adequately described by the direct effects of light availability and that the impacts of herbivores and their natural enemies, although also very important, appear to be largely independent of the plant's light environment, at least for short time periods comparable to the duration of my study.

Additional research, however, is needed to test the generality of these results in other locations and for other plant species because there remain good reasons to expect herbivore impact or bodyguard defense to vary among light environments in other situations (Chapter 4). But for *P. biflora*, the overall picture at the Wilson Botanical Garden is that plant performance is strongly dominated by the negative direct effects of shading and insect herbivores and the positive indirect effects of wasp bodyguards. The indirect effects of shading or ants had relatively little influence.

***Passiflora* herbivores and herbivory**

My studies of *P. biflora* provide the first experimental demonstration of the importance of insect herbivory on a *Passiflora* species, despite long-standing interest in the plant-herbivore interactions of this group. The exclusion of insect herbivores with insecticide significantly reduced herbivory and significantly increased all measures of plant growth and fruit production. Unexpectedly, however, almost all herbivory on *P. biflora* was caused by flea beetles, not by heliconiine caterpillars, despite the amount of prior attention given to this latter group. Thus, my results suggest that the impact of flea beetles may be a much underappreciated aspect of *Passiflora*-herbivore coevolution.

Effects of shading on herbivory

Variation in herbivore abundance or herbivory is commonly found across light availability gradients. Sometimes herbivore abundance or their damage is greater on plants in sunnier environments, sometimes greater in shaded environments, and sometimes no difference is found. In my studies of *P. biflora*, I found no evidence that heliconiine oviposition was influenced by shading, but flea beetles, heliconiine caterpillars, leaf miners, and whitefly pupae tended to be more abundant on either the plants in full sun or in 50% shade (Chapter 3). Nevertheless, I found little evidence that shading had much of an effect on insect herbivory despite these differences in herbivore abundance.

Possibly, insect herbivores on *P. biflora* are relatively insensitive to light-induced changes in plant quality because they are nearly all *Passiflora* specialists and, therefore, well adapted to the physical and chemical variation of their host plants. Therefore, light-induced gradients in herbivory may be less common in systems and habitats dominated by specialist herbivores.

Alternatively, in heterogeneous light habitats such as tropical forests, herbivores (and their natural enemies) may "spillover" from productive habitats to unproductive ones because their home ranges encompass many host plants, across a wide variety of light environments. In other words, insects in these habitats may be relatively blind to environmental heterogeneity, in contrast to the situation in more homogeneous habitats. This spillover phenomenon may be one of the ecological consequences of fine-scale patchiness in light availability found in many tropical forests.

Extrafloral nectaries

In my dissertation research, I also investigated the effect of shading on the number of EFNs per leaf. I believe this is the first time that this question has been addressed. Most previous investigators have considered EFN number to be a static plant trait, although plasticity in this trait could be adaptive in certain circumstances (i.e., if fitness costs are associated with having too many or two few EFNs for a particular light environment; Chapter 4). Therefore, I predicted that EFN number might respond to the light environment. At the whole plant level, sun plants certainly had more leaves than shaded plants, and therefore more EFNs overall, but at the per leaf level, the number of EFNs was unaffected by the plant's light environment. Therefore, for *P. biflora*, light availability appears to have no effect on number of EFNs per leaf. But the light environment may also affect nectar production by individual EFNs and, therefore, the plant's attractiveness to bodyguards. Further research is needed on this interesting but little studied topic.

Wasps, not ants

Before the start of my experiments with *P. biflora*, I expected that ants would be attracted to the numerous leaf EFNs and that, if ants serve as plant bodyguards, their exclusion (using Tanglefoot) should result in increased herbivore abundance and herbivory. But I found no evidence in my studies that ants negatively impacted butterfly oviposition, beetles, or any other herbivore group. I argue that ants were ineffective plant bodyguards because of their low abundance at the study site. Surprisingly, however, I detected that the exclusion of ants actually resulted in higher fruit production (Chapter 2) and higher whitefly pupae density (Chapter 3), indicating that ants may in certain circumstances have a negative effect on plant performance. These results solidly reject the hypothesis that ants serve a bodyguard role on *P. biflora* at the Wilson Botanical Garden, which conflicts with studies elsewhere, in which protective role of ants on *Passiflora* has been demonstrated. Similar negative impacts of ants may occur on other plants with EFNs, but it may be masked by their overall strong effect as plant bodyguards.

In contrast to ants, wasps appeared to have a strong impact on heliconiine butterfly eggs and caterpillars and, by extension, on plant performance. From a total of 345 butterfly eggs counted across both studies, I found a total of only 23 caterpillars, none of which reached the largest, most damaging instar (excluding *Dione juno*; Chapter 3). So, unlike ants, wasps appear to play a huge role in controlling the abundance of these specialist herbivores. Therefore, I predict that if wasps could be selectively excluded, *P. biflora* performance would suffer substantially due to release of these caterpillars, thus demonstrating a strong positive indirect effect of wasps as plant bodyguards.

Environmental conditionality of bodyguard defense

Although shaded plants had many fewer leaves than plants in full sun and, therefore, presumably less extrafloral nectar, I found no evidence that shaded plants were less attractive to potential plant bodyguards. There are several possible explanations for this result. Perhaps sample sizes were only sufficient to detect strong indirect effects, not weak ones, or maybe the experiments did not run long enough for the full impact of herbivores or natural enemies to be manifested. Given the strong top-down impact of wasps on heliconiine eggs and caterpillars and the tremendous potential for these caterpillars to defoliate plants, the slight oviposition preference of heliconiine butterflies for sun plants might result in substantially greater herbivore impact on these plants if wasp protection was somehow prevented, thereby demonstrating an indirect effect of shading on plant performance.

Future directions

There are several areas of research that I would like to pursue in future studies. First is the question of light-induced plasticity of leaf cyanogenesis. Cyanogenesis is probably the primary chemical defense of *P. biflora*, and there are good reasons to expect leaf cyanogenesis to respond to shading, but in this dissertation I was unable to measure differences among plants using an insensitive cyanogenesis assay (data not presented here). Although cyanogenesis is a common plant defense, very little is known about the environmental plasticity of this trait.

Second, I am very interested in pursuing the idea of light-induced plasticity in extrafloral nectar production by individual EFNs. There are good reasons to expect that more nectar is produced by sun plants than shaded plants (Chapter 3 and 4), but essentially nothing is known about this topic.

Third, my studies of *P. biflora* at the Wilson Botanical Garden suggest that ants may be largely parasites of an underappreciated plant-wasp mutualism. Other researchers have observed wasps visiting EFNs, but it has become almost dogmatic to assume that ants are the primary protectors of plants with EFNs. The potential importance of wasps as plant bodyguards needs to be tested experimentally, especially in places where both ants and wasps are abundant.

The ant-wasp turnover hypothesis

Although I observed relatively few ants on *P. biflora* at the Wilson Botanical Garden, other researchers have documented much higher ant abundances on this species, but in lowland habitats. Thus, the ineffectiveness of ants on *P. biflora* at the Wilson Botanical Garden may be simply a function of lower ant abundance at higher elevations. In lowland habitats, other researchers have noted low wasp parasitism of heliconiine butterfly eggs on *P. biflora*, whereas I found that wasps were the primary mortality agents for these insects. Thus, the apparent decline in parasitism at low elevations may be a direct consequence of the higher ant abundance that occurs there. I call this apparent replacement of ants with wasps as plant protectors at higher elevations the "Ant-Wasp Turnover Hypothesis" (Figure 5.1). This potential interaction between these natural enemies needs to be investigated further, because it may explain why plants in mid-elevation habitats have EFNs despite the low abundance of ants that occurs there, and why plant species restricted to high elevation habitats have lost their EFNs.

Final thoughts

Throughout my dissertation research, I have championed the idea that biotic interactions among trophic levels are important and that natural variation in these interactions should be expected. Many biologists are still reluctant to recognize the potential role of biotic interactions (other than competition) in determining the distribution and abundance of species or to deviate from the simple pair-wise study of species interactions. But I would argue that much progress in ecology will only be achieved by embracing this potential complexity and that an improved understanding of direct and indirect effects will be necessary to mitigate fully the environmental impacts of humans.

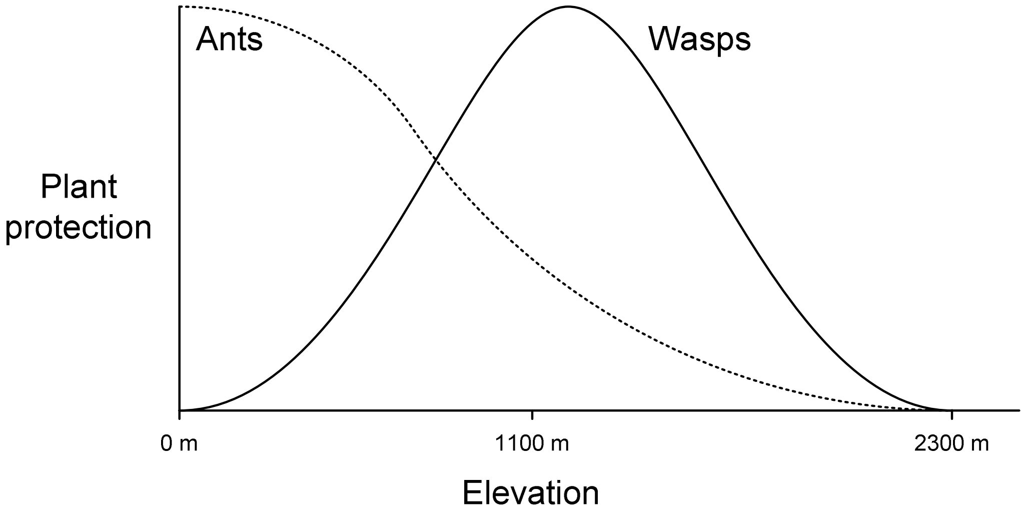


Figure 5.1. The ant-wasp turnover hypothesis. The Wilson Botanical Garden is located at ca. 1100 m elevation, where, based on this hypothesis, wasps are expected to be the primary plant bodyguards.