

IS ATTRACTION FATAL? THE EFFECTS OF HERBIVORE-INDUCED PLANT VOLATILES ON HERBIVORE PARASITISM

SARA J. OPPENHEIM¹ AND FRED GOULD

Department of Entomology, North Carolina State University, Raleigh, North Carolina 27695-8203 USA

Abstract. We investigated the relationship between parasitoid attraction to herbivore-induced plant volatiles and larval parasitism rates of two closely related heliothine, noctuid moths. *Heliothis subflexa* Guenee is a specialist on plants in the genus *Physalis*, while *Heliothis virescens* Fabricius is an extreme generalist. In North America, these species serve as the only known hosts for the specialist parasitoid *Cardiochiles nigriceps* Vierick; oviposition into *Helicoverpa zea*, a non-host, does occur but results in lethal encapsulation of *C. nigriceps*' eggs. *Heliothis virescens* larvae are parasitised by *C. nigriceps* far more frequently than are *H. subflexa* larvae. Parasitoid attraction to volatiles emitted by tobacco in response to herbivory by *H. virescens* has previously been demonstrated. Using field experiments, we examined the possibility that pre-detection defenses against parasitoid attraction to herbivore-induced plant volatiles are responsible for *H. subflexa*'s relatively low rates of parasitism by *C. nigriceps*.

Herbivore-damaged plants were significantly more attractive to *C. nigriceps* than were larvae, larval frass, larval saliva, or damaged leaves alone. Plant species affected parasitoid attraction: tobacco was the most preferred plant species, followed by *Physalis angulata*, and then cotton. The parasitoid was also more attracted to host species (*H. subflexa* and *H. virescens*) than to the non-host species, *H. zea*. There was an interaction between plant species and herbivore species: each plant species was most attractive when infested by its typical herbivore (e.g., *H. virescens* on tobacco). We compared these data with those of a previously published experiment on field parasitism of *H. virescens* and *H. subflexa*, conducted at the same time and place. Our results indicate that differences in parasitoid attraction to herbivore-induced plant volatiles do not contribute to *H. subflexa*'s relatively low parasitism rate. For the generalist, *H. virescens*, rates of attraction correspond with rates of parasitism; for the specialist, *H. subflexa*, they do not. Attraction to *H. subflexa* on *P. angulata* is greater than to *H. virescens* on *P. angulata*, yet parasitism of *H. subflexa* is much less than parasitism of *H. virescens*. These results indicate that pre-detection defenses are not responsible for *H. subflexa*'s low parasitism on *P. angulata*.

Key words: *Cardiochiles nigriceps*; *Heliothis*; herbivore defense; herbivore host range evolution; herbivore-induced plant volatiles; herbivore, specialist vs. generalist; natural enemies; parasitism rates; parasitoid; parasitoid attraction cf. increased parasitism; *Physalis angulata*; tritrophic interactions.

INTRODUCTION

The influence of natural enemies on the evolution of insect host range has been a point of debate for many years (Lawton and Strong 1981, Rhoades 1983). Natural enemies are a primary source of herbivore mortality (Strong and Larsson 1994, Müller and Godfray 1999), and parasitoids may be the most damaging of natural enemies (Hawkins et al. 1997). Herbivore vulnerability to natural enemies varies: in some systems, specialist herbivores, feeding on plants from a single genus or a single family, are better defended against natural enemies than are generalist herbivores (Smiley 1978, Denno et al. 1990, Ohsaki and Sato 1994). This observation has led some workers to suggest that the high proportion of specialists amongst insect herbivores (Menken 1995) may reflect selection to escape

natural enemies such as parasitoids (Bernays and Graham 1988, Hunter and Price 1992, Floyd 1996).

Herbivores defend themselves from parasitoids in a variety of ways. As described by Gross (1993), these defense strategies fall into three general categories: pre-detection defenses, such as reduction of cues that attract parasitoids (Vet and Dicke 1992, Steinberg et al. 1993) and shifts to less attractive host plants (Vinson 1984); post-detection defenses, such as noxious secretions and evasive maneuvers (Rank and Smiley 1994); and post-attack defenses, such as the encapsulation of parasitoid eggs or larvae (Strand and Pech 1995).

In some cases, pre-detection defenses are so effective that parasitoids are unable to detect either herbivores or their products (Takabayashi et al. 1995, Shimoda et al. 1997). Unfortunately for herbivores, parasitoids may circumvent these defenses by relying on herbivore-induced plant volatiles to locate hosts (Vet et al. 1991). Some parasitoids can determine from such volatiles alone whether the herbivore is at a vulnerable

Manuscript received 14 January 2002; revised 25 April 2002; accepted 27 April 2002.

¹ E-mail: Sara_Oppenheim@ncsu.edu

stage of development (Takabayashi et al. 1998). Other parasitoids use herbivore-induced volatiles to determine the species of herbivore attacking the plant, and visit only those plants infested by host species (Ageopoulos and Keller 1994, DeMoraes et al. 1998, Du et al. 1998).

Because parasitism is often lethal, we might expect strong selection on herbivores to overcome or compensate for parasitoid attraction to herbivore-induced volatiles. While the potential for these volatiles to increase the efficacy of natural enemies in controlling pest species has generated much interest (Cortesero et al. 2000), the relationship between herbivore-induced signals and herbivore parasitism rates is not well documented. Most workers have assumed that "The exploitation of plant-provided chemical cues by parasitoids can be expected to enhance parasitism rates" (Hoballah and Turlings 2001:554), but experimental evidence for this is lacking. In particular, the impact of herbivore-induced plant volatiles on parasitism of specialist vs. generalist herbivores has not been studied. To our knowledge, the role of induced volatiles in both attraction and parasitism (as opposed to attraction alone) has been examined in only one system. Thaler (1999), working with the generalist *Spodoptera exigua* and its parasitoid *Hyposoter exigua*, found that herbivore-induced plants were more attractive to parasitoids. Further, herbivores placed in holding cups near induced plants had higher rates of parasitism than those near uninduced plants (see Kessler and Baldwin [2001] for a discussion of the impact of induced volatiles on egg predation).

Thaler's (1999) findings support the hypothesis that an increase in parasitoid attraction leads to increased parasitism. Thus, specialists might reduce their vulnerability to natural enemies by using pre-detection defenses. However, Thaler's work involved only a generalist herbivore, and measured parasitism rates of caterpillars in holding cups rather than on host plants, limiting their ability to employ pre- or post-detection defenses. Thus, her results do not address the differential impact of herbivore-induced volatiles on parasitism of specialists and generalists in the field.

To explore how parasitoid attraction to herbivore-induced plant volatiles affects parasitism of specialist vs. generalist herbivores, we investigated the *Heliothis virescens* Fabricius–*Heliothis subflexa* Guenee–*Cardiochiles nigriceps* Vierick system. *Heliothis virescens* is an extreme generalist, feeding on the fruiting structures of at least 37 plant species in more than 14 different families (Sheck and Gould 1993), including tobacco, cotton, soybean, and other crops. *Heliothis subflexa* is monophagous, feeding only on plants in the genus *Physalis* (Laster 1972). *Physalis* produces fruits enclosed in a thin-walled, inflated calyx, which provides a structural refuge to *H. subflexa* (Sisterson and Gould 1999). While *H. virescens* does not typically occur on *Physalis*, second-instar and older larvae can

feed and grow on *Physalis* fruits (Oppenheim and Gould 2002). *Heliothis subflexa* and *H. virescens* differ primarily in their feeding habits, and are so closely related that mating between them produces fertile female offspring (Laster 1972).

Throughout much of the Southeastern United States, the specialist *Cardiochiles nigriceps* (Hymenoptera: Braconidae) is the predominant parasitoid of both *H. subflexa* and *H. virescens* (Lewis et al. 1967, Tingle and Mitchell 1982) and thus has a strong impact on the fitness of these species. Similarly, *C. nigriceps*, which can only develop in *Heliothis* spp. larvae (Lewis and Vinson 1971), is under strong selection to successfully locate and identify its hosts. Errors in host choice—such as oviposition into *Helicoverpa zea* (a close relative of *H. subflexa* and *H. virescens*)—are lethal for *C. nigriceps*.

Parasitism rates are much higher for *H. virescens* than for *H. subflexa*. For *H. virescens* on tobacco, parasitism varies from 25% (Johnson 1997) to 72% (Neunzig 1969). On cotton, rates as high as 96% have been observed (Lewis et al. 1972). For *H. subflexa* larvae on *Physalis angulata*, field collections consistently show much lower rates of parasitism: Roach (1975) found no parasitism, while Lewis et al. (1967) observed rates from 2% to 9% and Sisterson and Gould (1999) found 7% parasitism.

Post-detection defenses appear to play a strong role in the relative parasitism rates of *H. subflexa* and *H. virescens* (Oppenheim and Gould 2002). While both *H. subflexa* and *H. virescens* larvae feed on fruits enclosed within the calyx of *P. angulata*, *H. subflexa* larvae use this refuge more efficiently than *H. virescens* and experience much lower rates of parasitism on *P. angulata* than *H. virescens* larvae do. This, however, does not preclude the possibility that differences in pre-detection defenses against parasitoid attraction to herbivore-induced plant volatiles are also responsible for differences in parasitism of *H. subflexa* and *H. virescens*. Pre-detection defenses could explain *H. subflexa*'s low parasitism if *H. subflexa* larvae feed on *P. angulata* in a manner that reduces *C. nigriceps*'s ability to detect damaged plants. Jones et al. (1993), for example, found that the orientation of the leaves upon which a herbivore feeds can determine whether or not herbivore-induced plant volatiles are released. If *H. subflexa* larvae use strategic feeding to minimize volatile emissions from *P. angulata*, parasitoid attraction to *H. virescens* on *P. angulata* should be significantly greater than to *H. subflexa* on *P. angulata*, and this pre-detection defense could account for differential parasitism of *H. subflexa* and *H. virescens* on *P. angulata*.

Alternatively, differences in parasitism of *H. subflexa* and *H. virescens* might be unrelated to differences in parasitoid attraction to herbivore-induced plant volatiles. Instead, *H. subflexa*'s low parasitism may result from post-detection defenses alone.

To examine the possibility that pre-detection defenses are responsible for *H. subflexa*'s relatively low rates of parasitism by *C. nigriceps*, we tested *C. nigriceps*' attraction to a variety of plant-herbivore combinations. First, we confirmed that attraction depends upon herbivore-induced plant volatiles (DeMoraes et al. 1998) rather than upon larvae or larval products. Next, we examined the effect of different plant species, insect species, and combinations thereof on attraction of *C. nigriceps*. We compared attraction to host larvae (*H. subflexa* and *H. virescens*) with attraction to a closely related non-host, *H. zea*. Parasitoid attraction to commonly occurring plant-insect combinations (i.e., *H. virescens* on tobacco or cotton, *H. zea* on cotton, *H. subflexa* on *P. angulata*) was compared to uncommon combinations (i.e., *H. virescens* or *H. zea* on *P. angulata*, *H. subflexa* on tobacco). Finally, to determine whether *C. nigriceps*' attraction preferences are innate or learned, we investigated the responses of inexperienced female parasitoids to several plant-insect combinations.

MATERIALS AND METHODS

Insect strains and rearing

We obtained *Heliothis subflexa* larvae from a North Carolina State University (NCSU; Raleigh, North Carolina, USA) laboratory colony established using insects collected in Lenoir County, North Carolina in 1998. *Heliothis virescens* larvae were from an NCSU laboratory colony originally collected more than eight years ago. *Helicoverpa zea* larvae were from a USDA Agricultural Research Service laboratory colony maintained in Tifton, Georgia, USA. We reared individual larvae on corn-soy meal diet, as modified from Burton (1970). Prior to testing, all larvae were held in a rearing room under a L:D 14:10 regime. We used newly molted third-instar larvae for all treatments. Third-instar larvae of all three species tested fed on tobacco, cotton, and *Physalis angulata*, while earlier instars sometimes refused to feed, or died after feeding on a non-hostplant for 48 h.

Field tests of parasitoid attraction involved naturally occurring populations of *Cardiochiles nigriceps*. These parasitoid populations might previously have experienced *Heliothis virescens* and *Helicoverpa zea* on cotton, tobacco, and other locally available host plants, but were unlikely to have had prior experience with the *H. subflexa*-*P. angulata* combination (in repeated searches of the local area, we found no infested *P. angulata* plants). Tests of inexperienced-parasitoid's attraction involved laboratory-reared, mated female *C. nigriceps* from Dr. Glynn Tillman's laboratory colony in Tifton, Georgia, which had been in the laboratory for ~1 yr. Parasitoids were reared from diet-fed *H. virescens* larvae, and fed on sucrose. Female parasitoids were allowed to mate, but were not exposed to

plants, larvae, or larval products until the day of testing.

Plants

While several species of *Physalis* are acceptable to *H. subflexa*, larvae are most commonly found on *P. angulata* (Laster 1972, Yopez et al. 1990), and we used this species for all experiments. Our *P. angulata* plants were grown from seed collected in Orangeburg County, South Carolina, USA, in 1998. We planted seeds in flats, maintained them under greenhouse conditions until 5 cm high, and then transplanted seedlings into 8-L pots, which were large enough to allow plants to attain sizes typical of those found in the field. We used only *P. angulata* plants with >15 fruits. *Heliothis virescens*-susceptible tobacco seeds (variety: MacNair 373) were planted in flats, transferred to 8-L pots, and used at budding stage. Cotton seeds (strain: DPL 90) were started in flats, then transferred to 8-L pots, and only those with well-developed bolls were used. All plants were grown in a large, screened outdoor cage in Tift County, Georgia, and fertilized monthly with 2 g (one teaspoon) of Osmocote (NPK 14:14:14; Scotts, Marysville, Ohio). We transported plants to the field site as needed for tests, and discarded them after use in a single test.

Experimental site

We conducted field experiments in a 12-ha cotton field located in Irwin County, Georgia. Two rows of tobacco were planted along the midline of the cotton field; plants in each row were ~1 m apart. Tobacco plants were flowering when these tests were done, and were being fed on by naturally occurring *H. virescens* and *Manduca sexta* larvae; cotton plants ranged from small- to medium-boll stage, and were infested by *H. virescens* and *H. zea* larvae. Experimental plants were randomly interspersed with the field-grown tobacco.

Experimental methods

Experiment 1: Attraction to herbivore-damaged plants vs. other odor sources.—We provided four types of olfactory cues to searching parasitoids and measured parasitoid attraction to each. To test the effect of herbivore-induced plant volatiles, *H. subflexa* and *H. virescens* larvae were placed on intact *P. angulata* or tobacco plants and allowed to feed for 48 h before observation. We used a single plant, infested by two third-instar larvae, for each insect-plant combination. We used only plants with visible damage, and discarded plants after use in a single trial.

We tested the effect of olfactory cues such as damaged leaf volatiles, larval frass, and larval saliva from *H. subflexa* and *H. virescens* larvae feeding on *P. angulata* or tobacco leaves by placing larvae in cylindrical holding cups (8 cm wide, 5 cm tall) attached to 1.5-m stakes. Stakes were positioned beside *P. angulata* or tobacco plants so that holding cups were situated

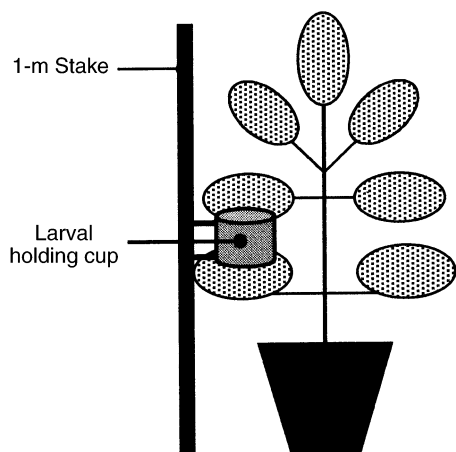


FIG. 1. Design of holding-cup arrangement used to test *Cardiochiles nigriceps*' attraction to larvae feeding on plants vs. larvae feeding in holding cups positioned in the plant's canopy. Larvae in holding cups were fed excised leaves or diet but could not damage the plant, so the plant released no systemic herbivore-induced volatiles.

in the middle canopy of the plant (Fig. 1). Holding cups were also used to test the effects of olfactory cues from herbivores feeding on diet (frass, saliva, and the diet itself) and olfactory cues from diet alone (control tests). Holding-cup treatments were set up 48 h in advance of observation, allowing time for leaf damage, feces, and saliva to accumulate. We kept all treatments in a large screened outdoor cage before testing. We conducted seven trials, two in August 1998, and five during June, July, and August 1999.

To evaluate parasitoid attraction, we recorded the number of visits by *C. nigriceps* to each treatment during a 2-h period of continuous observation. We defined a visit as directed searching of a plant (i.e., alighting on a plant; flying circles around a plant; multiple, sequential flights through the plant canopy). We observed treatments, rather than individual *C. nigriceps*; thus, the number of visits reflects discrete visitation events (i.e., wasp approaches, searches, and departs) rather than how many different individuals visited a treatment.

Statistical analysis.—We used an incomplete factorial design, blocked by date, with two plant levels (tobacco or *P. angulata*), three larva levels (*H. virescens*, *H. subflexa*, or none), and three feeding-site levels (on diet, on excised leaves, or on plant). The SAS procedure GLM (SAS Institute 1990) was used to conduct an analysis of variance on the number parasitoid visits. Data were square-root transformed before analysis. We treated block (reflecting climate variables, such as cloud cover and wind, which might affect parasitoid activity), plant, herbivore, feeding site, and treatment (i.e., the three-way plant–herbivore–feeding site interaction) as main effects, with block \times treatment interaction as the error term. Where main effects were significant we used Fisher's protected least-significant-dif-

ference procedure to make comparisons among least-squares means.

Experiment 2: Attraction to tobacco, cotton, and *P. angulata* damaged by host and non-host larvae.—To determine whether *C. nigriceps* is more attracted to some herbivore species, plant species, or particular plant–herbivore combinations than to others, we examined parasitoid attraction to larvae of *H. virescens*, *H. subflexa*, and *Helicoverpa zea* (a non-host for *C. nigriceps*) on three different plant species—tobacco, cotton, and *P. angulata*. Each plant species was subdivided into seven treatments: *Heliothis virescens*, *H. subflexa*, or *Helicoverpa zea* feeding on the plant; *Heliothis virescens*-, *H. subflexa*-, or *Helicoverpa zea*-damaged plant with larvae and damaged material removed; and an undamaged control. We used all seven treatments for each plant species, for a total of 21 different treatments per replicate. Treatments with larvae on the plant during observation exposed parasitoids to visual, local olfactory, and systemic olfactory cues associated with larval feeding; damaged plants with larvae and damaged material removed provided only systemic volatile cues.

We placed two third-instar larvae on each plant 48 h before field observation to allow damage to accrue, and used only plants with visible damage. For treatments involving *Heliothis subflexa* on cotton, we covered each plant with a nylon sleeve to prevent larvae from abandoning plants during the 48-h pre-experiment period; we removed these sleeves before field observation. After 48 h we collected larvae and damaged leaves from the appropriate plants, and covered sites of leaf abscission with aluminum foil to prevent volatile release from wounds. Infested and control plants were held in a screened outdoor cage prior to tests, then transported to the field for tests. Tests were conducted on 14 different days in July and August of 1999. As described in Experiment 1, we observed parasitoid attraction continuously for 2 h.

Statistical analysis.—We used a mixed-model factorial design, blocked by date, with three plant levels (tobacco, *P. angulata*, or cotton), four larva levels (*H. virescens*, *H. subflexa*, *Helicoverpa zea*, or none), and three damage levels (continuing damage from larva on plant, damaged but with larvae and damaged tissue removed, or undamaged). Plant, herbivore, and damage were treated as fixed effects, date as a random effect. Where main effects were significant, the interaction between herbivore species and plant species was included. The effects of plant–herbivore combination were analyzed separately using a mixed-model factorial design with two plant–herbivore combination levels (“common” = *Heliothis virescens*_{Tobacco}, *H. virescens*_{Cotton}, *Helicoverpa zea*_{Tobacco}, *H. zea*_{Cotton}, and *Heliothis subflexa*_{Physalis}; “uncommon” = *H. virescens*_{Physalis}, *Helicoverpa zea*_{Physalis}, *Heliothis subflexa*_{Tobacco}, *H. subflexa*_{Cotton}) blocked by date. Block effects, reflecting differences

between observations on different days, were included in both analyses. Where main effects were significant we used Fisher's protected least-significant-difference procedure to make comparisons among least-squares means.

Experiment 3: Inexperienced *Cardiochiles nigriceps*.—Mated, laboratory-reared, female *C. nigriceps* were tested three to four days after emergence, and had no prior experience with plants or larvae. However, to ensure that wasps were in a searching mode, we placed individual *C. nigriceps* in a 2-quart (1.816-L) container with two third-instar *H. subflexa* larvae immediately before conducting observations. Thus, tested wasps had been exposed to *H. subflexa* prior to experimentation, but had never experienced *H. virescens* or any of the plant species used. Immediately following the initiation period, we released individual *C. nigriceps* from containers placed on a 0.75-m-high platform in the center of a 3-m-diameter circle. Treatments consisting of *H. virescens* on tobacco, *P. angulata*, and cotton, and of *H. subflexa* on tobacco and *P. angulata*, were evenly spaced along the perimeter of this circle in random order. We placed two, third-instar larvae on each plant 48 h before observation. We used two plants per treatment, and used only plants with visible larval damage.

After release, we monitored parasitoid flight and alighting. We scored parasitoids as either making a choice (indicated by landing on a treatment) or flying away, and recorded the first treatment visited by each *C. nigriceps*.

We conducted all observations between 0800 and 1200, a peak searching time for field populations of *C. nigriceps* (G. Tillman, *personal communication*). We did experiments on three separate days in August 1999, and tested 40 female *C. nigriceps* on each day.

Statistical analysis.—We used a factorial design, blocked by date, with three plant levels (tobacco, cotton, or *P. angulata*), and two larva levels (*H. virescens* or *H. subflexa*). Block effects, plant effects, and herbivore effects were treated as main effects. Interaction terms for plant \times herbivore, plant \times block, and herbivore \times block were included after testing for significance of main effects. Where main effects were significant we used Fisher's protected least-significant-difference procedure to make comparisons among least-squares means. A Z test was used to test the null hypothesis that *C. nigriceps* displays no preference for tobacco vs. *P. angulata* (i.e., no effect of plant species). To assess this, we compared the overall frequency of tobacco choices (total visits to tobacco + *H. virescens* and tobacco + *H. subflexa*), *P. angulata* choices, and cotton choices.

RESULTS

Experiment 1: attraction to herbivore-damaged plants

Cardiochiles nigriceps strongly preferred plants being fed upon by larvae. In least-squares means tests,

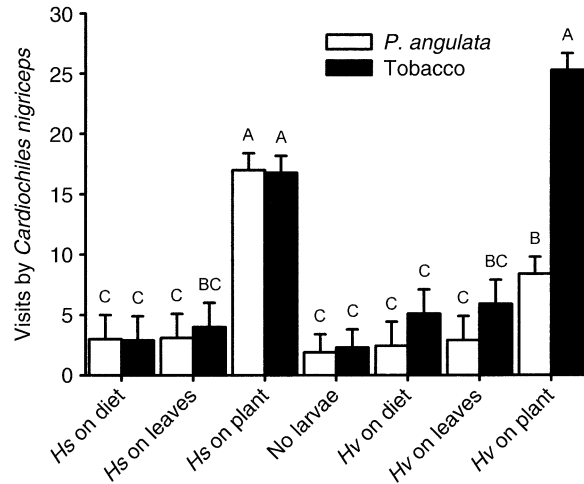


FIG. 2. Visits by natural populations of *Cardiochiles nigriceps* to various odor sources. The following were tested: *Heliothis subflexa* (Hs) or *H. virescens* (Hv) in holding cup, feeding on diet; *H. subflexa* or *H. virescens* in holding cup, feeding on excised *Physalis angulata* or tobacco leaves; holding cup containing diet but no larvae; *H. subflexa* or *H. virescens* on *P. angulata* or tobacco. Histogram bars with the same uppercase letter do not differ significantly between treatments at $P < 0.05$. Error bars represent +1 SE.

only those treatments with larvae feeding on plants differed significantly from controls (diet placed beside plant) in their attractiveness to *C. nigriceps* (Fig. 2; see also Table 1). Plants with larvae in holding cups feeding on plant leaves or diet were no more attractive than uninfested control plants.

Herbivore species did not affect the number of parasitoid visits, but plant \times herbivore effects were significant in the *Heliothis virescens* on plant treatments. While visits to *H. subflexa*-infested *Physalis angulata* and tobacco plants did not differ ($P = 0.8335$), *H. virescens*-infested tobacco was visited three times as often as *H. virescens*-infested *P. angulata* (25 ± 1.4 visits per two hours for infested tobacco; 8 ± 1.4 visits/2 h for infested *P. angulata* [means ± 1 SE]; $P < 0.0001$). The overall mean frequency of visits to *H. subflexa* treatments (9 ± 0.95 visits/2 h) was indistinguishable from that of visits to *H. virescens* treatments (10 ± 0.95 visits/2 h; $P = 0.7289$).

Experiment 2: attraction to tobacco, cotton, and *P. angulata* damaged by host and non-host larvae

Among treatments where larvae were feeding on plants at the time of observation, *H. virescens*_{Tobacco} was the most visited (43 ± 3.27 visits per two hours [mean ± 1 SE]; Fig. 3). *Heliothis subflexa*_{Tobacco} was the second most visited treatment (29 ± 3.27 visits/2 h) and differed at the $P = 0.0001$ level from all treatments except *H. subflexa*_{Physalis} (26 ± 3.27 visits/2 h; $P = 0.3009$). *Helicoverpa zea*_{Tobacco} (18 ± 3.27 visits/2 h) ranked fourth, but visits to other treatments with *H. zea* (*H. zea*_{Cotton} and *H. zea*_{Physalis}) did not differ significantly

TABLE 1. Statistical results for *Cardiochiles nigriceps* attraction of *Heliothis virescens* and *H. subflexa* feeding on tobacco plants, *Physalis angulata* plants, or on diet/plant material in holding cups placed beside plants.

Source of variation	df	Type III ss	MS	F	P
A) Whole model†					
Model	17		7.5478	11.63	<0.0001
Error	62		0.6492		
Corrected total	79				
B) Model components					
Herbivore	1	0.0623	0.0624	0.10	0.7576
Plant	1	0.6289	0.6289	0.97	0.3288
Feed site	4	79.8733	19.9683	30.76	<0.0001
Block	6	24.0842	4.0140	6.18	<0.0001
Herb × Plant × Site	5	7.3084	1.4617	2.25	0.0602

† $R^2 = 0.7612$, $cv = 33.5128$.

from visits to undamaged plants. Visits to *Heliothis virescens*_{Cotton} and *H. subflexa*_{Cotton} were similar in frequency to visits to undamaged control plants (Fig. 3A). The order of parasitoid preference was identical for all damaged treatments, regardless of whether larvae were feeding on the plant or had been removed, along with damaged tissue, prior to observation (Fig. 3B).

Plant species and herbivore species each had a significant effect on the number of visits to each treatment, and there was a significant interaction between them. Among the three plant species tested, tobacco was most visited (25 ± 2.47 visits/2 h across all tobacco treatments), followed by *P. angulata* (14 ± 2.47 visits/2 h) and cotton (7 ± 2.53 visits/2 h). In both the overall ANOVA model and in least-squares means tests the effect of plant species was significant at the $P < 0.0005$ level (Table 2).

Visits to different herbivore species reflected the status of larvae as hosts for *C. nigriceps*: *H. virescens* (22 ± 2.59 visits/2 h) and *H. subflexa* (20 ± 2.59 visits/2 h) were visited equally ($P = 0.1648$); the frequency of visits to treatments with *Helicoverpa zea* (11 ± 2.59 visits/2 h) was significantly less than visits to *Heliothis virescens* and *H. subflexa* ($P < 0.0001$ for both), and was statistically indistinguishable from visits to treatments with no larvae (7 ± 2.67 visits/2 h; $P = 0.0800$).

Plant-herbivore combinations defined as common (*H. virescens*_{Tob.}, *H. virescens*_{Cot.}, *Helicoverpa zea*_{Tob.}, *H. zea*_{Cot.}, and *Heliothis subflexa*_{Phy.}) were visited more frequently (22 ± 2.56 visits/2 h) than uncommon combinations (*H. subflexa*_{Tob.}, *H. subflexa*_{Cot.}, *H. virescens*_{Phy.}, and *Helicoverpa zea*_{Phy.}; 16 ± 2.66 visits/2 h; $P = 0.0032$), though both categories were more visited than undamaged control plants (8 ± 3.72 visits/

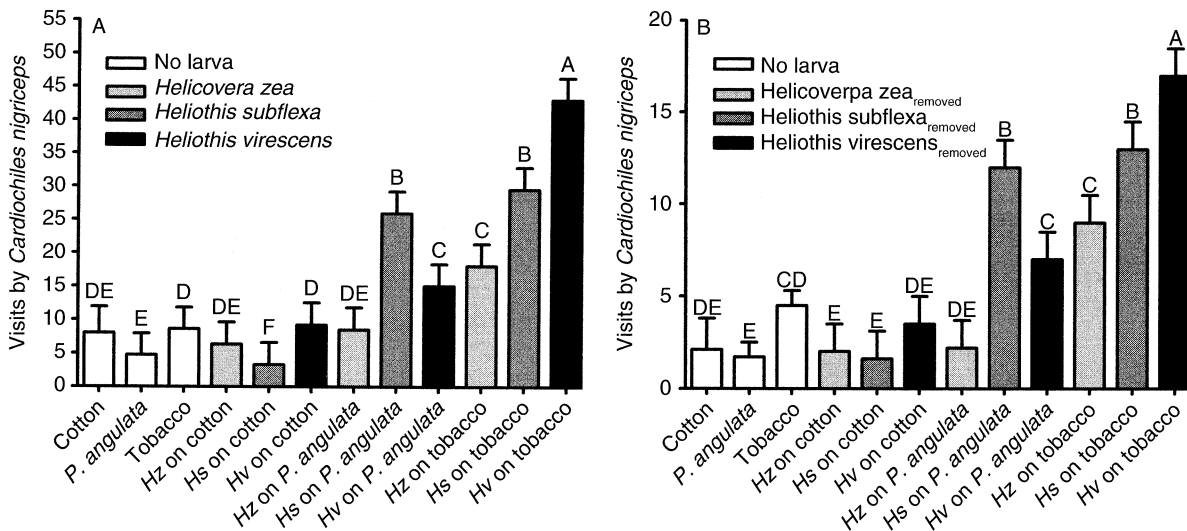


FIG. 3. Visits by natural populations of *Cardiochiles nigriceps* to various plant-herbivore combinations. The following were tested: undamaged cotton, *Physalis angulata*, or tobacco; *Helicoverpa zea*, *Heliothis subflexa*, or *Heliothis virescens* feeding on cotton, *P. angulata*, or tobacco; *H. virescens*-, *H. subflexa*-, or *Helicoverpa zea*-damaged cotton, *P. angulata*, or tobacco with larvae and damaged material removed. (A) Treatments with larvae feeding on plants at the time of observation; (B) Treatments with larvae and damaged tissue removed prior to observation. Histogram bars with the same uppercase letters do not differ significantly between treatments at $P < 0.05$. Error bars represent +1 SE.

TABLE 2. Statistical results for *Cardiochiles nigriceps* attraction to *Heliothis virescens*, *H. subflexa*, and *Helicoverpa zea* on tobacco, *Physalis angulata*, and cotton.

Source of variation	df	F	P
Herbivore	2, 104	18.88	<0.0001
Plant	2, 104	131.07	<0.0001
Damage	3, 42	1.58	0.2082
Herbivore × Plant	4, 104	16.50	<0.0001

Note: Values shown are for the Type III tests of fixed effects.

2 h; $P < 0.0001$ and 0.0055 , respectively) (Table 2). Visits to plants on which insects were feeding at the time of observation (18 ± 2.64 visits/2 h) were more frequent than visits to undamaged control plants ($P = 0.0004$), but did not differ significantly from visits to damaged plants with larvae and damaged tissue removed (17 ± 2.68 visits/2 h; $P = 0.0889$).

Experiment 3: inexperienced *Cardiochiles nigriceps*

Cardiochiles nigriceps females demonstrated a strong preference for tobacco, regardless of the herbivore infesting it (Fig. 4; $Z = 3.5857$, $P = 0.0002$). Tobacco visits were much more frequent than *P. angulata* visits: over half of the *C. nigriceps* tested flew directly to tobacco (10 ± 0.89 visits/2 h), while visits to *P. angulata* (4 ± 0.89 visits/2 h) were significantly less frequent ($P = 0.0010$). Cotton was the least visited plant species (2 ± 1.41 visits/2 h), and differed from tobacco ($P = 0.0011$), but not from *P. angulata* ($P = 0.2999$). Herbivore species had no effect on the number of visits, nor did the herbivore × block and plant × block interactions (Table 3). Herbivore × plant interactions were significant, however: *Heliothis virescens*_{Tobacco} was the most visited combination (12 ± 1.18 visits/2 h), *H. virescens*_{Cotton} the least visited (3 ± 1.18 visits/2 h).

DISCUSSION

Differences in larval parasitism of *Heliothis subflexa* and *H. virescens* cannot be explained by differences in *Cardiochiles nigriceps*' attraction to herbivore-induced

plant volatiles. In another experiment, run simultaneously in the same field as the parasitoid-attraction experiments described here, we exposed herbivore-infested plants to parasitoids for 12 h (Oppenheim and Gould 2002). Parasitism of *H. virescens* was much higher on tobacco than on *Physalis angulata* (42% vs. 15%), which in turn was greater than parasitism of *H. subflexa* on *P. angulata* (1%). These results are striking in comparison to our findings regarding parasitoid attraction to *H. virescens* on tobacco, *H. virescens* on *P. angulata*, and *H. subflexa* on *P. angulata*. For the generalist *H. virescens*, rates of parasitoid attraction correspond with rates of larval parasitism: on tobacco, attraction and parasitism are both very high; on *P. angulata*, both are intermediate. These findings are consistent with the results of Thaler (1999), who found a positive relationship between parasitoid attraction and parasitism rates for the generalist *Spodoptera exigua*.

For the specialist *H. subflexa*, however, we found no correspondence between attraction and parasitism. *Cardiochiles nigriceps* attraction to *H. subflexa* on *P. angulata* is much greater than to *H. virescens* on *P. angulata*, yet *H. subflexa* on *P. angulata* has the lowest rate of parasitism. In this system, parasitoid attraction rates are poor predictors of *H. subflexa*'s parasitism rate.

Our results allow us to reject pre-detection defenses as an explanation for *H. subflexa*'s low parasitism. Clearly, *P. angulata* produces herbivore-induced volatile signals that *C. nigriceps* can detect. *Physalis angulata*, in spite of being locally rare, was very attractive to *C. nigriceps* when attacked by herbivores. Cotton, though locally abundant and a common host of *H. virescens*, was the least attractive plant species overall. *P. angulata* was visited more than twice as often as cotton, while visits to tobacco were >4 times as frequent. This preference was conspicuous in both experienced, field-origin females and those reared in the laboratory (who had never encountered tobacco), suggesting that *C. nigriceps*' preference for tobacco over other plants is innate. It is unclear why cotton ranked so low in attractiveness; one possibility is that the fre-

TABLE 3. Statistical results for inexperienced *Cardiochiles nigriceps* attraction to *Heliothis subflexa* on *Physalis angulata* or tobacco and *H. virescens* on cotton, *P. angulata*, or tobacco.

Source of variation	df	Type III ss	MS	F	P
A) Whole model†					
Model	12		0.6887	74.57	0.0133
Error	2		0.0092		
Collected total	14				
B) Model components					
Herbivore	1	0.0002	0.0002	0.03	0.8876
Plant	2	5.7115	2.8558	309.21	0.0032
Block	2	0.5166	0.2583	27.97	0.0345
Herbivore × Plant	1	1.0193	1.0193	110.37	0.0089
Herbivore × Block	2	0.0319	0.0160	1.73	0.3666
Plant × Block	4	0.2471	0.0618	6.69	0.1343

† $R^2 = 0.9978$, $cv = 4.0202$.

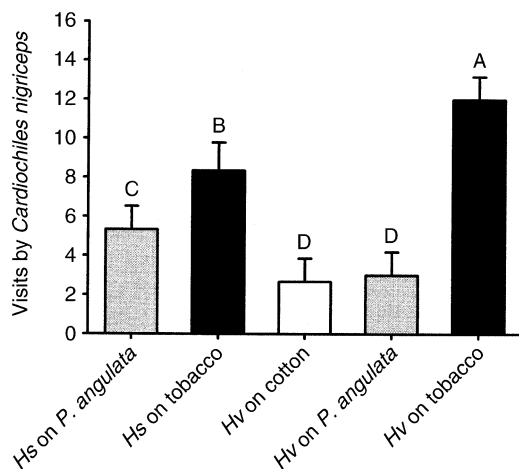


FIG. 4. Visits by inexperienced female *Cardiochiles nigriceps* to various plant-herbivore combinations. The following were tested: *Heliothis subflexa* (*Hs*) feeding on *Physalis angulata* or tobacco and *H. virescens* (*Hv*) feeding on cotton, *P. angulata*, or tobacco. Wasps' first choice after being released is shown. Histogram bars with the same uppercase letters do not differ significantly between treatments at $P < 0.05$. Error bars represent $+1$ SE.

quent occurrence of the non-host *Helicoverpa zea* on cotton makes cotton an unrewarding plant to search. We also found no evidence that *Heliothis subflexa*'s mode of feeding on *P. angulata* reduces *C. nigriceps*' ability to detect *H. subflexa*-infested plants—on the contrary, *P. angulata* plants damaged by *H. subflexa* were much more attractive than those damaged by *H. virescens* or *Helicoverpa zea*.

Cardiochiles nigriceps' ability to locate host herbivores did not depend in a simple fashion on plant species or herbivore species. Instead, the frequency of parasitoid visits depended on the particular plant-herbivore combination. For both tobacco and *P. angulata* (though not for cotton), we found that commonly occurring combinations of host herbivore and plant were more attractive than uncommon combinations: Attraction to *Heliothis virescens* on tobacco was greater than to *H. subflexa* on tobacco, and attraction to *H. subflexa* on *P. angulata* was greater than to *H. virescens* on *P. angulata*. This combination effect is especially striking because it indicates an apparent relationship between the three trophic levels. For parasitoids to distinguish between plants damaged by different species of herbivore, the signals associated with damage by particular herbivore attackers must differ in ways that parasitoids can detect. DeMoraes et al. (1998) found that differences in how tobacco responds to herbivory by *H. virescens* vs. *Helicoverpa zea* reflect herbivore identity: Volatiles released by *H. virescens*-damaged plants differ both qualitatively and quantitatively from those released by *H. zea*-damaged plants.

Whether similarly diagnostic differences in the volatiles induced by *H. virescens* and *H. subflexa* underlie

our observations of *C. nigriceps*' preference for particular plant-herbivore combinations is unclear. In the case of phylogenetically distant herbivores, diagnostic differences in herbivore saliva or feeding style—factors that affect volatile release—seem likely (e.g., Turlings et al. 1990). *Heliothis subflexa* and *H. virescens*, however, are close relatives, and a difference in plant response to these two species would be of considerable interest. Alternatively, differences in *C. nigriceps*' attraction may depend on quantitative differences in herbivore feeding: 3rd-instar *H. subflexa* feed less than *H. virescens* on tobacco. The fact that *H. subflexa*_{Tobacco} was the second-most-visited combination, however, argues against a simple quantitative explanation of differences in parasitoid attraction.

The reduction in parasitoid visits to *H. virescens* on *P. angulata* vs. tobacco suggests that this novel host plant-herbivore combination may provide some olfactory enemy-free space to *H. virescens* larvae (Bush 1975, Diehl and Bush 1989, Mopper et al. 1995). In addition, *C. nigriceps*' innate preference for tobacco might make any "not-tobacco" host plant a comparative refuge. The keen detection capabilities of parasitoids, however, suggest that they can successfully track their hosts over many plant species. The enemy-free space provided by novel combinations is therefore unlikely to persist over evolutionary time. Instead, our results indicate that, rather than escape parasitoids by moving onto an undetectable host plant, specialist herbivores like *H. subflexa* may employ post-detection defenses to reduce their vulnerability to parasitoids (Clancy and Price 1987, Damman 1987).

These findings suggest that the effects of plant induction on herbivorous insects may depend upon context (Dicke et al. 1990). Evaluation of the possibility that the release of herbivore-induced plant volatiles serves plants as a "call for help" (Sabelis and deJong 1988, Whitman and Eller 1990) will require scrupulous examination of how increased parasitoid attraction affects herbivores in various tritrophic systems. Similarly, caution should be employed in assessing the potential usefulness of plant induction in parasitoid-based control programs. In the *Heliothis subflexa*-*Heliothis virescens*-*Cardiochiles nigriceps* system, at least, the ecological impact of herbivore-induced plant volatiles depends upon the particular plant-herbivore combination rather than on the degree of parasitoid attraction. Parasitoid recruitment via herbivore-induced plant volatiles may have only a minor impact on overall mortality in the life history of specialized herbivores like *H. subflexa*.

ACKNOWLEDGMENTS

Grateful thanks to Glynn Tillman and Joe Lewis (USDA-ARS, Tifton, Georgia) for invaluable help in carrying out field experiments; to Consuelo DeMoraes (Penn State), for many helpful discussions of this work as it progressed; to Keith Hopper (USDA-ARS, Newark, Delaware) and George Kennedy (NCSU) for meticulous critical readings of this man-

uscript; to Cavelle Brownie (NCSU) for statistical input; and to Jing Wang for outstanding technical support. This work was supported by the Keck Foundation for Behavioral Biology and by a National Science Foundation Grant (4-45072) to F. Gould.

LITERATURE CITED

- Agelopoulos, N. G., and M. A. Keller. 1994. Plant-natural enemy association in the tritrophic system, *Cotesia rubecula*-*Pieris rapae*-Brassicaceae (Cruciferae). I. Sources of infochemicals. *Journal of Chemical Ecology* **20**:1725-1732.
- Bernays, E., and M. Graham. 1988. On the evolution of host specificity in phytophagous arthropods. *Ecology* **69**:886-892.
- Burton, R. L. 1970. A low-cost artificial diet for corn earworm. *Journal of Economic Entomology* **63**:1969-1970.
- Bush, G. L. 1975. Sympatric speciation in phytophagous parasitic insects. Pages 187-206 in P. W. Price, editor. *Evolutionary strategies of parasitic insects and mites*. Plenum Press, New York, New York, USA.
- Clancy, K. M., and P. W. Price. 1987. Rapid herbivore growth enhances enemy attack: sublethal plant defense remains a paradox. *Ecology* **68**:733-737.
- Cortesero, A. M., J. O. Stapel, and W. J. Lewis. 2000. Understanding and manipulating plant attributes to enhance biological control. *Biological Control* **17**:35-49.
- Damman, H. 1987. Leaf quality and enemy avoidance by larvae of a pyralid moth. *Ecology* **68**:87-97.
- DeMoraes, C. M., W. J. Lewis, P. W. Pare, and J. H. Tumlinson. 1998. Herbivore-infested plants selectively attract parasitoids. *Nature* **393**:570-573.
- Denno, R. F., S. Larsson, and K. L. Olmstead. 1990. Role of enemy-free space and plant quality in host-plant selection by willow beetles. *Ecology* **71**:124-137.
- Dicke, M., M. W. Sabelis, J. Takabayashi, J. Bruin, and M. A. Posthumus. 1990. Plant strategies in manipulating predator-prey interactions through allelochemicals: prospect for application in pest control. *Journal of Chemical Ecology* **16**:3091-3118.
- Diehl, S. R., and G. L. Bush. 1989. The role of habitat preference in adaptation and speciation. Pages 345-365 in D. Otte and J. A. Endler, editors. *Speciation and its consequences*. Sinauer Associates, Sunderland, Massachusetts, USA.
- Du, Y., G. M. Poppy, W. Powell, J. A. Pickett, L. J. Wadhams, and C. M. Woodcock. 1998. Identification of semiochemicals released during aphid feeding that attract the parasitoid *Aphidius ervi*. *Journal of Chemical Ecology* **24**:1355-1368.
- Floyd, T. 1996. Top-down impacts on creosotebush herbivores in a spatially and temporally complex environment. *Ecology* **77**:1544-1555.
- Gross, P. 1993. Insect behavioral and morphological defenses against parasitoids. *Annual Review of Entomology* **38**:251-273.
- Hawkins, B. A., H. V. Cornell, and M. E. Hochberg. 1997. Predators, parasitoids, and pathogens as mortality agents in phytophagous insect populations. *Ecology* **78**:2145-2152.
- Hoballah, M. E. F., and T. C. J. Turlings. 2001. Experimental evidence that plants under caterpillar attack may benefit from attracting parasitoids. *Evolutionary Ecology Research* **3**:553-565.
- Hunter, M. D., and P. W. Price. 1992. Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* **73**:724-732.
- Johnson, M. T. 1997. Interaction of resistant plants and wasp parasitoids of tobacco budworm (Lepidoptera:Noctuidae). *Environmental Entomology* **26**:207-214.
- Jones, C. G., R. F. Hopper, J. S. Coleman, and V. A. Krischik. 1993. Control of systemically induced herbivore resistance by plant vascular architecture. *Oecologia* **93**:452-456.
- Kessler, A., and I. Baldwin. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. *Science* **291**:2141-2144.
- Laster, M. L. 1972. Interspecific hybridization of *Heliothis virescens* and *H. subflexa*. *Environmental Entomology* **1**:692-687.
- Lawton, J. H., and D. R. Strong. 1981. Community patterns and competition in folivorous insects. *American Naturalist* **118**:317-338.
- Lewis, W. J., J. R. Brazzel, and S. B. Vinson. 1967. *Heliothis subflexa* a host for *Cardiochiles nigriceps*. *Journal of Economic Entomology* **60**:615-616.
- Lewis, W. J., A. N. Sparks, R. L. Jones, and D. J. Barras. 1972. Efficiency of *Cardiochiles nigriceps* as a parasite of *Heliothis virescens* on cotton. *Environmental Entomology* **1**:468-471.
- Lewis, W. J., and S. B. Vinson. 1971. Suitability of certain *Heliothis* (Lepidoptera:Noctuidae) as hosts for the parasite *Cardiochiles nigriceps*. *Annals of the Entomological Society of America* **64**:970-972.
- Menken, S. B. J. 1995. Evolution of insect-plant interactions. *Proceedings Experimental and Applied Entomology*, N.E.V. Amsterdam **6**:3-15.
- Mopper, S., M. Beck, D. Simberloff, and P. Stiling. 1995. Local adaptation and agents of selection in a mobile insect. *Evolution* **49**:810-815.
- Müller, C. B., and H. C. J. Godfray. 1999. Predators and mutualists influence the exclusion of aphid species from natural communities. *Oecologia* **119**:120-125.
- Neunzig, H. H. 1969. The biology of the tobacco budworm and the corn earworm in North Carolina. North Carolina Experimental Station Technical Bulletin Number **196**.
- Ohsaki, N., and Y. Sato. 1994. Food plant choice of *Pieris* butterflies as a trade-off between parasitoid avoidance and quality of plants. *Ecology* **75**:59-68.
- Oppenheim, S. J., and F. Gould. 2002. Behavioral adaptations increase the value of enemy-free space for *Heliothis subflexa*, a specialist herbivore. *Evolution* **56**:679-689.
- Rank, N. E., and J. T. Smiley. 1994. Host-plant effects on *Parasyrphus melanderi* (Diptera: Syrphidae) feeding on a willow leaf beetle *Chrysomela aeneicollis* (Coleoptera: Chrysomelidae). *Ecological Entomology* **19**:31-38.
- Rhoades, D. F. 1983. Herbivore population dynamics and plant chemistry. Pages 155-220 in R. F. Denno and M. S. McClure, editors. *Variable plants and herbivores in natural and managed systems*. Academic Press, New York, New York, USA.
- Roach, S. H. 1975. *Heliothis* spp. larvae and associated parasites and diseases on wild hostplants in the Pee Dee area of South Carolina. *Environmental Entomology* **4**:725-728.
- Sabelis, M. W., and M. C. M. deJong. 1988. Should all plants recruit bodyguards? Conditions for a polymorphic ESS of synomone production in plants. *Oikos* **53**:247-252.
- SAS Institute. 1990. *SAS Procedures Guide-Version 6 edition*. SAS Institute, Cary, North Carolina, USA.
- Sheck, A. L., and F. Gould. 1993. The genetic basis of host range in *Heliothis virescens*: larval survival and growth. *Entomologia Experimentalis et Applicata* **69**:157-172.
- Shimoda, T., J. Takabayashi, W. Ashihara, and A. Takafuju. 1997. Response of predatory insect *Scolothrips takahashii* toward herbivore-induced plant volatiles under laboratory and field conditions. *Journal of Chemical Ecology* **23**:2033-2048.
- Sisterson, M. S., and F. Gould. 1999. The inflated calyx of *Physalis angulata*: a refuge from parasitism for *Heliothis subflexa*. *Ecology* **80**:1071-1075.

- Smiley, J. 1978. Plant chemistry and the evolution of host specificity: new evidence from *Heliconius* and *Passiflora*. *Science* **201**:745–747.
- Steinberg, S., M. Dicke, and L. M. Vet. 1993. Relative importance of infochemicals from first and second trophic level in long-range host location by the larval parasitoid *Cotesia glomerata*. *Journal of Chemical Ecology* **19**:47–59.
- Strand, M. R., and L. L. Pech. 1995. Immunological basis for compatibility in parasitoid–host relationships. *Annual Review of Entomology* **40**:31–56.
- Strong, D. R., and S. Larsson. 1994. Is the evolution of herbivore resistance influenced by parasitoids? Pages 261–276 in B. A. Hawkins and W. Sheehan, editors. *Parasitoid community ecology*. Oxford University Press, Oxford, UK.
- Takabayashi, J., Y. Sato, M. Horikoshi, R. Yamaoka, S. Yano, N. Ohsaki, and M. Dicke. 1998. Plant effects on parasitoid foraging: differences between two tritrophic systems. *Biological Control* **11**(2):97–103.
- Takabayashi, J., S. Takahashi, M. Dicke, and M. A. Posthumus. 1995. Developmental stage of herbivore *Pseudaletia separata* affects production of herbivore-induced synomone by corn plants. *Journal of Chemical Ecology* **21**:273–287.
- Thaler, J. S. 1999. Jasmonate-inducible plant defenses cause increased parasitism of herbivores. *Nature* **399**:686–688.
- Tingle, F. C., and E. R. Mitchell. 1982. Effect of synthetic pheromone on parasitization of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) in tobacco. *Environmental Entomology* **11**:913–916.
- Turlings, T. C. J., J. H. Tumlinson, and W. J. Lewis. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* **250**:1251–1253.
- Vet, L. E. M., and M. Dicke. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology* **37**:141–172.
- Vet, L. E. M., F. L. Waeckers, and M. Dicke. 1991. How to hunt for hiding hosts: the reliability/detectability problem in foraging parasitoids. *Netherlands Journal of Zoology* **41**(2–3):202–213.
- Vinson, S. B. 1984. How parasitoids locate their hosts: a case of insect espionage. Pages 325–348 in T. Lewis, editor. *Insect communication*. Academic Press, London, UK.
- Whitman, D. W., and F. J. Eller. 1990. Parasitic wasps orient to green leaf volatiles. *Chemoecology* **1**:69–75.
- Yepez, F. F., J. Clavijo, and I. Romero. 1990. Especies del complejo *Heliothis virescens* (Fabricius, 1977) (Lepidoptera: Noctuidae) y sus plantas hospederas en Venezuela. *Review Facultad Agronomy (Maracay)* **16**:169–175.