Journal of Insect Behavior, Vol. 16, No. 4, July 2003 (© 2003)

Comparison of Host-Searching and Ovipositional Behavior of *Cardiochiles nigriceps* Viereck (Hymenoptera: Braconidae), a Parasitoid of *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae), in Tobacco and Cotton

P. Glynn Tillman[1–3] and Benjamin G. Mullinix, Jr.[2]

Accepted April 2, 2003; revised June 1, 2003

To compare host-searching and ovipositional behavior of Cardiochiles nigriceps *Viereck (CN) in tobacco and cotton, behavior of CN females was observed on* Hellothis virescens *(Febricius)-infested plants and recorded using a Psion Organizer II with Observer software. Hover, search, oviposit, preen, rest, walk, agony-search, drop-search, and miss-search were the nine behaviors observed for each plant species. Agony-search was a searching behavior exhibited when CN females were prevented from ovipositing in a host because they were unable to reach the host. Drop-search and miss-search were the searching behaviors that occurred by CN females after a host dropped from the plant and when CN females missed ovipositing in a host, respectively. Hovering, searching, oviposition, miss-search, resting, and walking were equally common for CN on tobacco and cotton. Agony-search was exhibited more on tobacco than cotton, while drop-search and preening occurred more on cotton than tobacco. Female CN spent more time hovering in tobacco than in cotton, indicating that these females preferred tobacco to cotton. Female parasitoids also spent more time in the main ovipositional sequence, hover–search–oviposit, in tobacco than in cotton, mainly because females hovered more in tobacco than in cotton. Frequency and time of behavioral sequences*

[1]USDA, ARS, Crop Protection and Management Research Laboratory, P.O. Box 748, Tifton, Georgia 31794.
[2]University of Georgia, Experimental Statistics, P.O. Box 748, Tifton, Georgia 31794.
[3]To whom correspondence should be addressed. e-mail: pgt@tifton.usda.gov.

0892-7553/03/0700-0555/0 © 2003 Plenum Publishing Corporation

associated with experiencing frustration in ovipositing due to difficulty in reaching or finding a host and subsequent level of success in ovipositing in a new host were determined. Overall, the rate of successful oviposition by CN females was significantly higher in tobacco (87.7%) than in cotton (79.5%). For cotton and tobacco, the rate of successful oviposition by CN females was significantly reduced (ca. 40.0%) when they experienced frustration in ovipositing compared to the rate of ovipositional success (100%) for CN females when hosts were present and easily accessible. Encountering ovipositional frustration also increased the duration of time for successful oviposition in comparison to the time spent for problem-free oviposition in cotton and tobacco. Interestingly, with successful oviposition, the amount of time spent in a behavioral sequence was not significantly different for tobacco and cotton regardless of whether females experienced ovipositional frustration or not. However, female CN invested more time in tobacco than in cotton when they were unsuccessful in ovipositing due to the inaccessibility or disappearance of hosts. CN females' preference for tobacco over cotton probably gave them more motivation to continue searching for hosts, especially those not easily attained, on tobacco than on cotton. Nevertheless, CN females readily searched in cotton for HV even in the presence of host-infested tabacco. CN females possibly could be successful in maintaining levels of HV below an economic threshold when using a tobacco trap crop to protect cotton as the main crop.

KEY WORDS: *Cardiochiles nigriceps*; *Heliothis virescens*; parasitoid; host-searching behavior; tobacco; cotton.

INTRODUCTION

Cardiochiles nigriceps Viereck (CN) is a solitary endoparasitoid that can contribute substantially to the biological control of the tobacco budworm, *Heliothis virescens* (Fabricius) (HV). This parasitoid also parasitizes *Heliothis subflexa0* (Guenée) on ground cherry, Physalis spp. (Oppenheim and Gould, 2000a) and larvae of the beet armyworm, *Spodoptera exigua* (Hübner) (Ruberson *et al.*, 1994). Since *H. subflexa* is not very common in nature, and the beet armyworm is a rare host for this wasp, CN basically is host specific for HV and usually is associated with HV on all host plants of this pest.

Larvae of HV can be found in tobacco, *Nicotiana tabacum* L., in very high numbers, especially in early season, and appears to prefer tobacco to cotton, *Gossypium hirsutum* L. (Neunzig, 1969; Fitt, 1989; Manley *et al.*, 1991). CN is an important parasitoid of HV on tobacco (Chamberlin and Tenhet, 1926; Roach, 1976; Tingle and Mitchell, 1982; Johnson and Manley, 1983; Bidlack *et al.*, 1991; Jackson *et al.*, 1996) and cotton (Lewis *et al.*, 1972; Manley *et al.*, 1991). High mortality (50–100%) of HV by CN can occur in

these crops. According to the previous authors, both tobacco and cotton are suitable for HV and CN immature development and survival.

Even though HV is a generalist feeder and can infest a wide range of host plants, it feeds primarily on one to four host plants at any moment in time, depending on seasonal availability and phenological states of host plants. This seasonal succession of host plants varies by geographical location. Thus, in Georgia, the primary host plants for HV in the spring include toadflax, *Linaria canadensis* (F.) Dumont (Barber, 1937), Carolina cranesbill, *Geranium carolinianum* L. (Snow *et al.*, 1966), and crimson clover, *Trifolium incarnatum* L. (Pair, 1994). Japanese honeysuckle, *Lonicera japonica* Thunb. (Pair, 1994), is an important spring and fall host plant. Tobacco is a host plant in late spring (Barber, 1937). Summer host plants include tobacco and cotton (Barber, 1937). The main fall host plants are beggarweed, *Desmodium purpureum* (P. Mill.) Fawcett and Rendle (Chamberlin and Tenhet, 1926; Barber, 1937; Snow and Burton, 1967; Jackson *et al.*, 1984), and postharvest tobacco (Barber, 1937; Jackson *et al.*, 1984). Interestingly, tobacco is available as a host plant for HV for a longer period of time than any other host plant.

Behavior of CN searching for HV on host plants includes hovering, searching, and oviposition. Once a foraging female locates a host plant, she hovers around the plant, sometimes staying over one spot close to the plant and then moving around the plant (Chamberlin and Tenhet, 1926). Hovering is similar to flying except the overall movement of the wasp is generally slower and wing movement is more intense. Vinson (1975) described hovering (referred to as scanning) behavior in detail and provided a photograph of CN exhibiting this behavior. The predominant cues that elicit hovering behavior may be plant volatiles emitted by damaged plants (De Moraes *et al.*, 1998). When a female hovers near a host-damaged site on the plant, she typically lands on the plant and begins searching for the host. Searching behavior involves antennating the plant until the female finds the host. Antennation behavior can be described as "drumming" the antennae close to the plant. Antennectomy removes the ability of CN to find a host, so antennation is considered essential for searching in HV (Hays and Vinson, 1971). Vinson and Lewis (1965) observed that CN would follow a "trail" to the host while antennating the plant, and Vinson (1968) later reported that a substance from the mandibular glands of the host elicited this searching response. Although antennae are the primary organs involved in host finding by CN, the ovipositor is necessary for final acceptance (laying an egg) of a host. The mandibular secretion stimulates antennation and can elicit ovipositional thrusting, but a second factor associated with the cuticle appears to be necessary for oviposition (Hays and Vinson, 1971). When a HV host is located, a CN female antennates the larva, positions herse over the

larva, inserts her ovipositor into this larva, and deposits an egg into the host (Chamberlin and Tenhet, 1926).

Host defenses can affect the host-searching behavior of parasitoids. Host defenses have been categorized by Gross (1993) into three types: (1) avoidance and concealment defenses by the host, (2) behavioral and morphological defenses of the host, and (3) physiological defenses of the host against endoparasitoids. Type 2 defenses were further categorized into five groups: (1) morphological traits, (2) evasive behaviors such as vigorous wiggling, (3) aggressive behaviors such as biting, (4) protection by attending ants, and (5) parental care. These host defenses can be very efficient in deterring parasitoids trying to attack them. Utilizing Type 1 defenses, Heliothis subflexa can complete entry into the thin-walled inflated calyx surrounding a fruit of the ground cherry within 5 min, leaving very little time for CN to locate them (Oppenheim and Gould, 2002a). When HV larvae use a head swipe to apply an oral exudate to attacking CN females, the parasitoids become agitated and begin to preen (Hays and Vinson, 1971). Moreover, CN females reject hosts painted with this oral exudate.

Plant species and plant volatiles also affect the host-searching behavior of female CN. Since CN females will hover and land on tobacco plants in the absence of their insect hosts (Vinson, 1975), they are very attracted to the plant itself. Herbivory causes plants to release volatiles that differ qualitatively and/or quantitatively from volatiles associated with artificial damage (Whitman and Eller, 1990; Turlings *et al.*, 1990; Mattiaci *et al.*, 1994). In field choice bioassays, CN females demonstrated a significant preference for plant volatiles released from tobacco and cotton plants damaged by HV in comparison to those released by undamaged plants of tobacco and cotton (Vinson, 1975; De Moraes and Lewis, 1999). Also, CN preferred tobacco to cotton when exposed to undamaged plants, volatiles from damaged plants with no HV, and damaged plants with HV (Oppenheim and Gould, 2002b).

The success of a parasitoid in maintaining levels of a pest species below an economic threshold using a trap crop to protect the main crop depends on the (1) attractiveness of the parasitoid to the trap crop, (2) ability of the parasitoid to maintain a high mortality of the pest in the trap crop so the trap crop will become a sink for the pest, and (3) ability of the parasitoid to shift between the trap crop and the main crop to ensure that any hosts that may exist in the main crop will also be parasitized. Tobacco can serve as a trap crop for HV in a cotton field (Tillman, 1999), and mortality by natural enemies, CN and a HV ascovirus, can reach very high levels (P. G. Tillman, unpublished). This study was conducted to compare host-searching behavior of this parasitoid in tobacco and cotton as part of a research project to assess

the potential of a strip of tobacco next to a cotton field to provide aid in enhancing this parasitoid in cotton.

MATERIALS AND METHODS

Insects

HV larvae were obtained from the USDA, ARS, Crop Protection and Management Research Laboratory in Tifton, GA. The colony originated from larvae collected from tobacco in 1997. Larvae were fed a laboratory bean diet (Perkins *et al.*, 1973) and held in a climatic-controlled room at 25°C, 14:10 LD, and 70% RH. Even though CN prefer to parasitize first instars (Chamberlin and Tenhet, 1926), newly ecdysed second instars were used as hosts in these behavioral studies because they were easier to observe than first instars, they were less likely to be killed than first instars when we handled them, and CN females have no difficulty parasitizing second instars. Behavioral studies with CN had to be conducted in the field with feral females because laboratory-reared CN either escaped or spent most of their time trying to escape from small cages in the laboratory, large field cages, and greenhouses.

Plants

Tobacco (strain K-326) and cotton (variety DP 5415) plants were grown in 2.8-L plastic nursery pots (one plant/pot) in the greenhouse at 25–30°C and 50–70% RH, with a photoperiod of 16:8 LD. Osmocote 14-14-14 (Scotts–Sierra Horticulture Products Co., Marysville, OH) was used as a time-release fertilizer. Plants were 8–9 weeks old (pinhead square stage for cotton and vegetative stage for tobacco) when used in experiments. Both species were between 75 and 90 cm high. In the field in south Georgia, female HV are attractive to the plant stages we used in the test even though higher oviposition occurred in tobacco when the two plant species were present together in the same field (Tillman, 1999).

Experimental Procedure

Five potted plants of each plant species were transferred to the middle of a 5-ha cotton field containing feral CN females. Three HV early second instars per plant were placed in the terminal of each potted plant, and all of them began feeding on these plants within 5 to 10 min. During the 2 h HV were allowed to feed before the test, CN females were prohibited from

searching on the plants. After this 2-h feeding period, the potted plants were arranged randomly by plant species in a row, with 60 cm between pots. Then host-searching and ovipositional behaviors of CN females in tobacco and cotton were observed and recorded using a Psion Organizer II, Model LZ64 (Psion PLC, London, England), with Observer software (Noldus Information Technology, Inc., Sterling, VA). The test was repeated on 3 days with new plants and hosts. Each test was conducted around 0900 to 1500 h since ovipositional activity is much greater during that time of day (Chamberlin and Tenhet, 1926). Observation of females occurred for 4 h for 1 day and 3 h for 2 days, for a total of 10 h of observations. A single female was observed until she left the test arena, and then another female was observed. Once we observed that a host was parasitized, it was removed, and an unparasitized host was placed in the same position that the parasitized one occupied previously. Using a Nikon SM2-2T dissecting microscope (Nikon Instruments, Inc., Melville, NY), hosts observed to be parasitized during the test were dissected in the laboratory to verify oviposition. During a test if a host dropped to the ground, a new one was placed in the same position that the host that dropped had held.

Detailed descriptions of hovering, searching, oviposition, preening, resting, and walking behavior previously reported (Chamberlin and Tenhet, 1926; Hays and Vinson, 1971; Vinson, 1975) represent our observations of these behaviors. Host-searching that resulted in a successful oviposition in tobacco and cotton was referred to simply as searching. Since agony-search, drop-search, and miss-search were observed behaviors that had not been previously reported, these behaviors are described in detail later in this paper. Type II host defenses such as head-whipping that occurred during a positive oviposition event on tobacco and cotton were considered unsuccessful, and thus a female's response to them was not recorded separately but lumped in search behavior.

Statistical Analyses

Chi-square analyses were used to compare frequencies of individual host-searching behaviors of CN on cotton and tobacco. Unequal-n unequal-variance t tests were used to compare times of individual behaviors of CN on cotton and tobacco (Steel and Torrie, 1960). Times of behavioral sequences associated with oviposition in cotton and tobacco were compared using t tests. Frequencies of sequences of behaviors associated with difficulty in reaching and finding hosts were compared using chi-square analyses for cotton and tobacco, while t tests were used to compare times spent in these behavioral sequences on cotton and tobacco.

RESULTS AND DISCUSSION

Host-Searching and Ovipositional Behaviors

Hovering, searching, oviposition, preening, walking, and resting were host-searching behaviors observed in both tobacco and cotton. Preening, resting, and walking were behaviors not directly involved in host-searching, but they occurred intermittently during the host-searching process and thus were included in the overall activity. Sometimes a female would hover after searching and then return to searching, but hovering always preceded searching, and searching always preceded oviposition. When a female found a host while host-searching, she quickly stopped and antennated the host. Then she oviposited in the host while positioning her body away from the head of the host to avoid being bitten. Laboratory dissections of hosts observed to be parasitized during the test revealed that each field observation of oviposition resulted in an egg being laid in the host. Even though CN females do not avoid hosts previously parasitized by the same female for the first 5 days following oviposition (Hays and Vinson, 1971), most female wasps in this test oviposited in a host only once since hosts were removed soon after being parasitized. Only once, in tobacco, was a female observed to oviposit in a previously parasitized host soon (11 s) after the initial oviposition. After oviposition, a female continued to search the same plant for another host, stopped searching and hovered elsewhere over the same plant, stopped searching to preen, or moved to a new plant. After successfully or unsuccessfully attacking a host, a CN female generally walked a short distance away from the host and began preening, a behavior that involved cleaning the mouth, wings, and resting to recuperate from the attack. Sometimes after preening or resting, a CN female would feed on the extrafloral nectaries of cotton. Since vegetative tobacco does not have extrafloral nectaries or flowers, feeding was never observed on this plant species. After preening, a CN female rested on the plant, continued to search on the same plant, or moved to another plant.

Type I and Type II host defenses provided protection for HV against female parasitoids searching for hosts. Type I host defenses, avoidance and concealment, were evident when the young HV moved quickly into terminal leaves to feed and then became concealed in the terminal. Upon detecting a host hidden in a terminal, a CN female used her ovipositor as a probe to try stabbing the host in the terminal. However, even though a female would stretch her ovipositor far into the terminal, she sometimes was unable to reach the host with her ovipositor. Sometimes concealed HV would be further enabled to avoid CN attack by vigorous wiggling, a Type II host defense, which either pushed the host further into the hiding place or frightened the

CN female away. Agony-search was defined as searching behavior exhibited when CN females were unable to reach a host because a female encountering this problem became progressively agitated by her lack of success in ovipositing into the host. In her agitated state she exhibited one to all of several behaviors including running around in circles, biting the plant, and fluttering her wings, and as the number of unsuccessful attempts to oviposit increased, these behaviors increased in intensity.

Hosts dropping from the plant was another evasive Type II host defense strategy. When oviposition by a CN female was unsuccessful because the host dropped from the plant, the fate of the host was not studied because host-searching behavior of the females had to be observed continuously. However, in one case, a female was observed to fly down to a host that had fallen onto the leaf below its prefall position and parasitize this host. Drop-search refers to the searching that occurred by a CN female after a host dropped from the plant.

Aggressive behavior, another Type II host defense, in HV involved whipping the head back and forth and trying to bite attacking CN. Larger HV can actually bite the female parasitoid and stop searching activity. However, the most common result of a successful head-whipping defense in this study of smaller HV was that the female missed inserting the ovipositor into the host. Miss-search refers to the searching that occurred when CN females missed ovipositing in a host.

Considering individual host-searching behaviors of CN, neither tobacco nor cotton showed consistent preference for all nine behaviors (Table I; first group). Placing behaviors 5, 6, and 7 into a group and all other behaviors into a second group revealed that hovering, searching, oviposition, miss-search, resting, and walking were equally common for CN on tobacco and cotton. Thus, behaviors 5, 6, and 7 were the only ones that exhibited any differences in frequency between plant species. Agony-search was exhibited more on tobacco than cotton while drop-search and preening occurred more on cotton than tobacco.

Morphological differences between cotton and tobacco accounted for these differences in frequencies of individual agony-search and drop-search behaviors on these plant species. In vegetative tobacco, the terminal leaves are somewhat bundled together so a HV larva feeding inside the terminal could be inaccessible to CN females. A host feeding in the terminal of pin-head square cotton was more exposed to CN females than hosts in tobacco terminals since cotton terminal leaves branch out as they grow. Hosts did not drop as often in tobacco as in cotton for two reasons. First, hosts generally fed inside the terminals of tobacco where they could not drop very easily. Second, the foliage of tobacco was very sticky, and HV larvae were unable to detach themselves from the leaves very quickly.

Table I. Frequency of Individual Host-Searching Behaviors of *C. nigriceps* on Cotton and Tobacco

	Cotton		Tobacco		Total	
Behavior (No.)	*n*	Frequency (%)	*n*	Frequency (%)	*n*	Frequency (%)
Hover (1)	870	49.9	953	54.4	1823	52.2
Search (2)	447	25.6	428	24.5	1875	25.0
Oviposit (3)	103	5.9	130	7.4	233	6.6
Miss-search (4)	7	0.4	10	0.6	17	0.5
Agony-search (5)	4	0.2	20	1.1	24	0.7
Drop-search (6)	34	2.0	3	0.2	37	1.1
Preen (7)	161	9.2	95	5.4	256	7.3
Rest (8)	77	4.4	74	4.2	151	4.3
Walk (9)	42	2.4	38	2.2	80	2.3
Total by crop	1745	49.9	1751	50.1	3496	100

Behavior group comparison	Behaviors	χ^2	df	*P*
1st	1–9	61.8	8	<0.01
2nd	1–4, 8, 9	5.7	5	>0.3
3rd	5, 6	35.1	1	<0.01

Since tobacco has sticky foliage, we expected that the occurrence of preening would be higher on tobacco than on cotton. However, the opposite was observed. Preening was a response to either an ovipositional attack or a feeding event. Female parasitoids foraged for food after searching for hosts over a period of time. Food from extrafloral nectaries was only available on cotton, and female CN always preened before and after feeding from this food source. Thus, the higher occurrences of preening in cotton over tobacco appear to be due to differences in food availability and not to differences in degree of stickiness of the plant foliage. This conclusion is substantiated by the fact that the time for CN females to recuperate (preen and rest) from an ovipositional attack was similar for each plant species.

Female CN spent more time hovering in tobacco than in cotton, providing further evidence that these females preferred tobacco to cotton (Table II). They also spent twice as much time (not frequency) in drop-search in tobacco than in cotton. Even though mean agony-search time numerically was twice as long in tobacco as in cotton, the means for these two plants were not significantly different, probably due to the high variance. Minimum/maximum values for agony-search, however, suggest that a difference in time did occur for this behavior between the two plants. We concluded that CN females tended to be willing to invest more time trying to find HV in tobacco than in cotton. CN females could oviposit in host larvae equally quickly (3–4 s) on tobacco and cotton. Preening, resting, and walking times

Table II. Time of Individual Host-Searching Behaviors of *C. nigriceps* on Cotton and Tobacco

Behavior (No.)	Time (s) in Cotton		Time (s) in tobacco		Cotton vs. tobacco		
	n	Mean (SE)	*n*	Mean (SE)	Difference	SE	$\|t\|^a$
Hover (1)	870	7.9 (0.2)	953	11.6 (0.4)	−3.7	0.3	8.3***
Search (2)	447	13.4 (0.8)	428	11.7 (0.9)	1.7	0.9	1.4 NS
Agony-search (3)	4	61.8 (6.9)	20	128.7 (18.7)	−66.9	30.2	1.6 NS
Drop-search (4)	34	25.3 (3.5)	3	59.0 (9.0)	−33.7	8.5	2.8**
Miss-search	7	12.9 (3.8)	10	19.0 (5.0)	−6.1	4.8	0.9 NS
Oviposit	103	3.9 (0.2)	130	4.2 (0.2)	−0.3	0.2	1.0 NS
Preen	161	14.9 (1.8)	95	16.3 (2.4)	−1.4	2.1	0.5 NS
Rest	77	15.4 (2.5)	74	19.3 (3.0)	−4.0	2.8	1.0 NS
Walk	42	11.8 (2.8)	38	8.7 (1.5)	3.0	2.2	1.0 NS
Feed on nectar	40	24.8 (7.3)	0				
Comparisons of interest							
3 vs. 4							
Cotton					36.5	5.2	4.7**
Tobacco					69.7	13.9	3.4**

[a] unequal-*n*, unequal-variance *t*- test (Steel and Torrie, 1960).
** $P < 0.001$.
*** $P < 0.0001$.

were also the same on both plants. More time was spent in agony-search than in drop-search for both plants. A host sometimes responded to an approaching CN female by wiggling and dropping from the plant, and in most cases the host used its silk to make a "rope" to hang by and later return to the leaf from which it had dropped. Unless the wiggling of the host frightened the wasp away, a female parasitoid usually searched the area from which the host had dropped for a short period of time, but she soon determined that a host was no longer present, and she stopped searching the area. However, she would expend a lot of time and energy continuing to oviposit in an inaccessible host, probably because she still was able to detect its presence even though she could not touch it. Occurrence of searching on a plant and length of time spent on the plant by CN females have been shown to be higher on plants with hosts feeding on the plant than on plants with no host feeding or on plants with only the systemic volatiles induced by host feeding being released from the plants (Vinson, 1975; P. G. Tillman, unpublished). This indicates that the presence of host-seeking stimulants keeps the female focused on searching for hosts on the plant. Thus, the presence of these host-seeking stimulants and perhaps also vibrations of the host itself appeared to give a CN female the incentive to continue searching a little longer for an inaccessible host.

Sequences of Host-Searching Behavior

Establishing behavioral sequences associated with oviposition in cotton and tobacco revealed that most often hovering and then searching preceded oviposition (Table III). In one case, for each plant species, the female parasitoid oviposited in one host and then oviposited in a second host shortly after parasitizing the first host. However, even in that case, the females hovered and then searched before the first oviposition and searched between the oviposition events. Since the hover–search–oviposit behavioral sequence generally resulted in oviposition, we referred to it as the ovipositional sequence. Female parasitoids spent more time in the ovipositional sequence in tobacco than in cotton, mainly because the females hovered more over tobacco than cotton since they preferred tobacco to cotton.

The ultimate behavioral response of a female parasitoid to agony-search, drop-search, and miss-search was one of the following: (1) oviposition in the same host, (2) oviposition in a new host on the same plant, or (3) no oviposition on the present plant and changing to another crop or plant species (Table III). Ovipositing in the same host occurred twice after agony-search in cotton and tobacco, once after drop-search in cotton, and not at all

Table III. Time of Behavioral Sequences Associated with Oviposition in Cotton and Tobacco for *C. nigriceps*

	Time (s) in cotton		Time (s) in tobacco		Cotton vs. tobacco		
Behavior sequence	*n*	Mean (SE)	*n*	Mean (SE)	Difference	SE	|*t*|
Hover, search, oviposit[a]	90	21.4 (1.8)	126	34.1 (2.5)	−12.7	2.2	4.1***
Oviposit, search, oviposit[a]	1	6	1	10	—	—	—
Oviposit, change crop[b]	75	36 (9.6)	78	40.2 (9.4)	−3.7	13.4	0.3 NS
Agony, oviposit same host[c]	2	70 ± 0	2	86 (12.0)	−16.0	17.0	0.7 NS
Agony, oviposit in new host[c]	2	78 ± 0	10	169.4 (40.5)	−91.4	57.3	1.1 NS
Agony, change crop[c]	2	99.5 (3.5)	9	243.2 (36.6)	−143.7	26.1	3.9*
Drop, change crop[d]	23	45.0 (8.8)	3	93.7 (4.8)	−48.7	7.2	4.8***
Drop, oviposit new host[d]	13	58.2 (58.7)	0				
Drop, oviposit same host[d]	1	13.0	0				
2 drops, oviposit in new host[d]	1	32.0	0				
Miss, change crop[e]	5	17.2 (4.8)	8	18.3 (6.8)	−1.1	5.9	0.1 NS
Miss, oviposit in new host[e]	2	70.5 (28.5)	2	13.5 (2.5)	57	20.3	2.0 NS

[a]Sequence = two behaviors before oviposition + oviposition; time for all three behaviors.
[b]Sequence = oviposition, concluding behavior; time from oviposition up to concluding behavior.
[c]Sequence = agony-search, concluding behavior; time from agony-search up to concluding behavior.
[d]Sequence = drop-search, concluding behavior; time from drop-search to concluding behavior.
[e]Sequence = miss-search, concluding behavior; time from miss-search to concluding behavior.
* $P < 0.01$.
*** $P < 0.0001$.

after miss-search. A female always quit searching for a missed host in both cotton and tobacco. She may have considered that the host she missed was parasitized, or she was too frightened to try to parasitize the host again. Oviposition in a new host occurred in response to agony-search and miss-search in cotton and tobacco. Only in cotton did CN females respond to drop-search by ovipositing in another host on the same plant. The few drop-searches that did occur in tobacco resulted in the female CN leaving tobacco to search for hosts in cotton. The time for the agony-changed crop sequence and the drop-changed crop sequence was about twice as long in tobacco as in cotton. Thus, the female parasitoids were less likely to leave tobacco than cotton when they couldn't oviposit. However, the time for the oviposition-changed crop sequence was equal for tobacco and cotton. So, CN readily moved from tobacco to cotton and vice versa after a successful oviposition.

Host defenses affected the ovipositional success of CN. If a female CN was unable to oviposit due to a host dropping from the plant or an inability to reach a host, she was considered to have experienced frustration in ovipositing. We established behavioral sequences associated with the possibility of a female CN experiencing such frustration in oviposition with a current or first host (yes for frustrated and no for not frustrated) and subsequent oviposition in a new host (yes for oviposition and no for failure to oviposit). Then, frequency and time of behavioral sequences associated with frustration with ovipositing in a current host and subsequent level of success in ovipositing in a new host were determined. Overall, the rate of success for oviposition by CN females was significantly higher in tobacco (87.7%) than in cotton (79.5%) (Table IV). In part, the rate of ovipositional success for CN females was related to parasitoid attraction, for parasitization was higher in the preferred plant, tobacco, over the nonpreferred host plant, cotton. Similarly, Oppenheim and Gould (2002b) determined that CN attraction to HV on tobacco was much greater than to HV on ground cherry, and the highest rate of parasitization occurred on tobacco, the preferred plant. In our study, the lower success in cotton also may partially be influenced by the higher occurrence of host drops in cotton than in tobacco. For cotton and tobacco, the rate of success for oviposition by CN females was significantly reduced (ca. 40.0%) when they experienced frustration in ovipositing compared to the rate of success (100%) for CN females when they did not experience any hindrance in ovipositing. Encountering frustration in ovipositing also increased the duration of time for oviposition necessary for a successful oviposition in comparison to the time spent for problem-free oviposition on cotton and tobacco (Table V). The amount of time spent in a behavioral sequence associated with frustration was the same with or without oviposition, so CN females may allocate only a certain amount of time trying to oviposit upon encountering frustration in ovipositing. Interestingly, when

Table IV. Frequency of Behavioral Sequences Associated with Frustration (FR) in Ovipositing in a Current Host (CH) and Subsequent Level of Success in Ovipositing (OV) in a New Host (NH) for *C. nigriceps*

Sequence		Frequency in crop	
Frustration (CH)	Oviposition (NH)	Cotton	Tobacco
No	No	0	0
No	Yes	99	127
Yes	No	30	20
Yes	Yes	17	13
Comparison of interest			
No FR vs. FR			
Cotton		0, 99 (100.0% success rate)	30, 17 (36.2% success rate)
χ^2		79.5***	
Tobacco		0, 127 (100.0% success rate)	20, 13 (39.4% success rate)
χ^2		87.9***	
Cotton vs. tobacco			
All OV vs. no OV		116, 30 (79.5% overall success rate)	143, 20 (87.7% overall success rate)
χ^2		3.9*	

* $P < 0.01$.
*** $P < 0.0001$.

CN females were successful in ovipositing, the amount spent in a behavioral sequence was not significantly different for tobacco and cotton regardless of whether or not the females experienced frustration in ovipositing. However, female parasitoids stayed on tobacco longer than on cotton when they were unsuccessful in ovipositing in a host due to difficulty in reaching or finding a host. Overall, CN females invested more time in tobacco than in cotton. CN females definitely preferred tobacco to cotton, and preliminary data suggest that CN females also prefer frass from HV larvae feeding on tobacco to frass from this host feeding on cotton (P. G. Tillman, unpublished). Thus, CN females probably have more motivation to continue searching for HV larvae, even those not easily attained, in tobacco over cotton.

SUMMARY

CN females were highly attracted to tobacco, and the rate of ovipositional success of CN females was high in tobacco (87.7%) and cotton (79.5%). Even though CN females invested more time in tobacco, they readily searched in cotton for HV even in the presence of host-infested tobacco.

Table V. Time in Behavioral Sequences Associated with Frustration (FR) in Ovipositing in a Current Host (CH) and Subsequent Level of Success in Ovipositing (OV) in a New Host (NH) for *C. nigriceps*

Sequence		Time (s) in crop, mean (SE)	
Frustration (CH)	Oviposition (NH)	Cotton	Tobacco
No	Yes	24.8 (3.2)	33.9 (5.8)
Yes	No	46.1 (5.6)	143.7 (11.9)
Yes	Yes	59.4 (7.4)	98.0 (26.6)
Comparison of interest		Difference between means, SE, $\|t\|$	
OV: No FR vs. FR		−34.6, 8.1, 4.3**	-64.1, 27.2, 2.4*
FR: No OV vs. OV		−13.3, 9.3, 1.4 NS	45.7, 29.1, 1.6 NS
Cotton vs tobacco			
N/Y sequence		−9.1, 6.7, 1.4 NS	
Y/N sequence		−97.6, 13.2, 7.4**	
Y/Y sequence		−38.6, 26.7, 1.4 NS	

*$P < 0.01$.
*$P < 0.001$.

So CN females possibly could be successful in maintaining levels of HV below an economic threshold when using a tobacco trap crop to protect cotton as the main crop.

REFERENCES

Barber, G. W. (1937). Seasonal availability of food plants of two species of *Heliothis* in eastern Georgia. *J. Econ. Entomol.* **30:** 150–158.

Bidlack, D. S., Grant, J. F., and Pless, C. D. (1991). Seasonal incidence of larvae of the tobacco budworm and corn earworm, and their parasitoids, on tobacco in eastern Tennessee. *J. Agr. Entomol.* **8:** 155–162.

Chamberlin, F. S., and Tenhet, J. N. (1926). *Cardiochiles nigriceps* (Vier.) an important parasite of the tobacco budworm, *Heliothis virescens* (Fab.). *J. Agr. Res.* **33:** 21–27.

De Moraes, C. M., and Lewis, W. J. (1999). Analysis of two parasitoids with convergent foraging strategies. *J. Insect Behav.* **12:** 571–583.

De Moraes, C. M., Lewis, W. J., Paré, P. W., Albom, H. T., and Tumlinson, J. H. (1998). Herbivore-infested plants selectively attract parasitoids. *Nature* **393:** 570–573.

Fitt, G. P. (1989). The ecology of *Heliothis* species in relation to agroecosystems. *Annu. Rev. Entomol.* **34:** 17–52.

Gross, P. (1993). Insect behavioral and morphological defenses against parasitoids. *Annu. Rev. Entomol.* **38:** 251–273.

Hays, D. B., and Vinson, S. B. (1971). Acceptance of *Heliothis virescens* (F.) (Lepidoptera, Noctuidae) as a host by the parasite *Cardiochiles nigriceps* Viereck (Hymenoptera, Braconidae). *Anim. Behav.* **19:** 344–352.

Jackson, D. M., Tingle, F. C., and Mitchell, E. R. (1984). Survey of *Heliothis* spp. larvae found on Florida beggarweed and postharvest tobacco in Florida. *Fla. Entomol.* **67:** 130–141.

Jackson, D. M., Nottingham, S. F., Schlotzhauer, W. S., Horvat, R. J., Sisson, V. A., Stephenson, M. G., Foard, T., and McPherson, R. M. (1996). Abundance of *Cardiochiles nigriceps*

(Hymenoptera: Braconidae) on *Nicotiana* species (Solanaceae). *Biol. Control* **25:** 1248–1255.

Johnson, A. W., and Manley, D. G. (1983). Parasitism of tobacco budworm in South Carolina. *J. Ga. Entomol. Soc.* **18:** 1–6.

Lewis, W. J., Sparks, A. N., Jones, R. L., and Barras, D. J. (1972). Efficacy of *Cardiochiles nigriceps* as a parasite of *Heliothis virescens* on cotton. *Environ. Entomol.* **1:** 468–471.

Manley, D. G., DuRant, J. A., Johnson, A. W., and Roof, M. E. (1991). The tobacco budworm/bollworm complex (Lepidoptera: Noctuidae) and its parasites on field crops in South Carolina. *J. Agrl. Entomol.* **8:** 169–178.

Mattiaci, L., Dicke, M., and Posthumus, M. A. (1994). Induction of parasitoid attracting synomone in Brussels sprout plants feeding on *Pieris brassicae* larvae: Role of mechanical damage and herbivore elicitor. *J. Chem. Ecol.* **20:** 2229–2247.

Neunzig, H. H. (1969). The biology of the tobacco budworm and the corn earworm in North Carolina. Technical Bulletin 196. North Carolina Agricultural Experiment Station, Raleigh.

Oppenheim, S. J., and Gould, F. (2002a). Behavioral adaptations increase the value of enemy free space for *Heliothis subflexa*, a specialist herbivore. *Evolution* **56:** 679–689.

Oppenheim, S. J., and Gould, F. (2002b). Is attraction fatal? The effects of herbivore-induced plant volatiles on herbivore parasitism. *Ecology* **83:** 3416–3425.

Pair, S. D. (1994). Japanese honeysuckle (Caprifoliaceae): Newly discovered host of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae). *Environ. Entomol.* **23:** 906–911.

Perkins, W. D., Jones, R. L., Sparks, A. N., Wiseman, B. R., Snow, J. W., and McMillan, W. W. (1973). Artificial diets for mass rearing the corn earworm (Heliothis zea). production Research Report No. 154. USDA, ARS, Washington, DC.

Roach, S. H. (1976). *Heliothis* sp. and their parasites and diseases on crops in the Pee Dee region of South Carolina. ARS-S-111. USDA, ARS, Washington, DC.

Ruberson, J. R., Herzog, G. A., Lambert, W. R., and Lewis, W. J. (1994). Management of the beet armyworm (Lepidoptera: Noctuidae) in cotton: Role of natural enemies. *Fla. Entomol.* **77:** 440–453.

Snow, J. W., and Burton, R. L. (1967). Seasonal occurrence of the *Heliothis* complex on *Desmodium purpureum* with observations on parasitism by *Cardiochiles nigriceps. J. Ga. Entomol. Soc.* **2:** 47–52.

Snow, J. W., Hamm, J. J., and Brazzel, J. W. (1996). *Geranium carolinianum* as an early host for *Heliothis zea* and *H. virescens* (Lepidoptera: Noctuidae) in the southeastern United States, with notes on associated parasites. *Ann. Entomol. Soc. Am.* **59:** 506–509.

Steele, R. G. D., and Torrie, J. H. (1960). *Principles and Procedures of Statistics*, McGraw–Hill, New York.

Tillman, P. G. (1999). Tobacco as a trap crop for the tobacco budworm. In Herzog, G. A., Hardee, D. D., Ottens, R. S., Burris, G., and Ottea, J. A. (eds.), *Proceedings Beltwide Cotton Conferences, 3–7 January 1999, Orlando, FL*, National Cotton Council, Memphis, TN, pp. 1201–1203.

Tingle, F. C., and Mitchell, E. R. 91982). Effect of synthetic pheromone on parasitization of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) in tobacco. *Environ. Entomol.* **11:** 913–916.

Turlings, T. C. J., Tumlinson, J. H., and Lewis, W. J. (1990). Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* **250:** 1251–1253.

Vinson, S. B. (1968). Source of a substance in *Heliothis virescens* (Lepidoptera: Noctuidae) that elicits a searching response in its habitual parasite *Cardiochiles nigriceps* (Hymenoptera: Braconidae). *Ann. Entomol. Soc. Am.* **61:** 8–10.

Vinson, S. B. (1975). Biochemical coevolution between parasitoids and their hosts. In Price, P. W. (ed.), *Evolutionary strategies of Parasitic Insects and Mites.* Plenum, New York, pp. 14–48.

Vinson, S. B., and Lewis, W. J. (1965). A method of host selection by *Cardiochiles nigriceps. J. Econ. Entomol.* **58:** 869–871.

Whitman, D. W., and Eller, F. J. (1990). Parasitic wasps orient to green leaf volatiles. *Chemoecology* **1:** 69–75.