

OLFACTORY RESPONSES OF *Plutella xylostella* NATURAL ENEMIES TO HOST PHEROMONE, LARVAL FRASS, AND GREEN LEAF CABBAGE VOLATILES

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Abstract—The parasitoids *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae) and *Cotesia plutellae* (Hymenoptera: Braconidae), and the predator *Chrysoperla carnea* (Neuroptera: Chrysopidae), are potential biological control agents for the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). We present studies on the interactions between these bioagents and various host-associated volatiles using a Y olfactometer. *T. chilonis* was attracted to a synthetic pheromone blend (Z11–16:Ald, Z11–16:Ac, and Z11–16:OH in a 1:1:0.01 ratio), to Z11–16:Ac alone, and to a 1:1 blend of Z11–16:Ac and Z11–16:Ald. *C. plutellae* responded to the blend and to Z11–16:Ac and Z11–16:Ald. Male and female *C. carnea* responded to the blend and to a 1:1 blend of the major components of the pheromone, although no response was elicited by single compounds. Among the four host larval frass volatiles tested (dipropyl disulfide, dimethyl disulfide, allyl isothiocyanate, and dimethyl trisulfide), only allyl isothiocyanate elicited significant responses in the parasitoids and predator, but *C. plutellae* and both sexes of *C. carnea* did respond to all four volatiles. Among the green leaf volatiles of cabbage (*Brassica oleracea* subsp. *capitata*), only Z3–6:Ac elicited significant responses from *T. chilonis*, *C. plutellae*, and *C. carnea*, but *C. plutellae* also responded to *E2*–6:Ald and Z3–6:OH. When these volatiles were blended with the pheromone, the responses were similar to those elicited by the pheromone alone, except for *C. carnea* males, which had an increased

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response. The effect of temperature on the response of the biological agents to a mixture of the pheromone blend and Z3-6:Ac was also studied. *T. chilonis* was attracted at temperatures of 25–35°C, while *C. plutellae* and *C. carnea* responded optimally at 30–35°C and 20–25°C, respectively. These results indicate that the sex pheromone and larval frass volatiles from the diamondback moth, as well as volatile compounds from cabbage, may be used by these natural enemies to locate their diamondback moth host.

Key Words—Olfactory responses, *Trichogramma chilonis*, *Cotesia plutellae*, *Chrysoperla carnea*, *Plutella xylostella*, Lepidoptera: Yponomeutidae, sex pheromones, larval frass, green leaf volatiles, cabbage.

INTRODUCTION

Semiochemicals help natural enemies locate and recognize their hosts or prey (Vinson, 1985; Lewis and Martin, 1990; Vet and Dicke, 1992). Therefore, knowledge of the nature of these chemicals and their functional roles is important in the design of programs that use parasitoids and predators as biological control agents. The diamondback moth (DBM), *Plutella xylostella*, is one of the most serious pests of cruciferous crops throughout the world (Talekar and Shelton, 1993). All stages of the DBM are attacked by numerous parasitoids and predators, the former being the most widely studied. Polyphagous egg parasitoids, *Trichogramma* and *Trichogrammatoidea*, have received attention in the development of strategies to control DBM (Tabone et al., 1999); *Trichogramma chilonis* Ishii is reported to be particularly effective (Miura and Kobayashi, 1998). Larval parasitoids are the most predominant and efficient, particularly those belonging to two major genera *Diadegma* and *Cotesia* (*Apanteles*). *C. plutellae* Kurdjumov (Hymenoptera: Braconidae) is effective in lowland areas of the tropics and subtropics where it is the only larval parasitoid that can survive (Talekar and Shelton, 1993; Verkerk and Wright, 1996; Liu et al., 2000). In this context, *C. plutellae* is a good candidate for the biological control of DBM (Talekar and Shelton, 1993). Lacewings, *Chrysoperla* spp., are one of the most effective general predators of soft-bodied insects feeding on eggs and young larvae (New, 1988), *Chrysoperla carnea* being important for corn borers and aphids. This predator and *C. plutellae* have been used in an integrated pest management program to control DBM in cabbage (Reddy and Guerrero, 2000a).

We have conducted experiments to establish activity of DBM sex pheromone and larval frass volatiles, as well as green leaf volatiles (GLVs) from cabbage, on natural enemies of the pest. To our knowledge, this is the first time that such a study has been carried out. The main components of the DBM pheromone are (Z)-11-hexadecenal (Z11-16:Ald) and (Z)-11-hexadecenyl acetate (Z11-16:Ac) (Chow et al., 1977; Tamaki et al., 1977). An 8:2 to 4:6 blend of them was highly attractive to males in the field (Koshihara et al., 1978). Addition of 1%

(*Z*)-11-hexadecenol (Z11-16:OH) increased captures of males (Koshihara and Yamada, 1980). GLVs from cabbage, *Brassica oleracea* var. *capitata* L., are 1-hexanol, (*Z*)-3-hexen-1-ol, 1-hexen-3-ol, hexanal, (*E*)-2-hexenal, hexyl acetate, and (*Z*)-3-hexenyl acetate (Reddy and Guerrero, 2000b). The larval frass volatile of DBM is a mixture of dipropyl disulfide, dimethyl disulfide, methyl propyl disulfide, and dimethyl trisulfide (Auger et al., 1989).

METHODS AND MATERIALS

Insects. *T. chilonis* were reared on eggs of DBM in 250-ml beakers under a 16L:8D photoperiod, $28 \pm 1^\circ\text{C}$, and 60–65% relative humidity. Adults of *T. chilonis* emerged eight days after the eggs had been parasitized. They were collected daily, transferred to glass tubes (10 cm long \times 2.5 cm ID), and supplied with a 20% honey–water solution. Females used for experiments were 3-days old, mated, and without previous oviposition experience. *C. plutellae* were reared on DBM larvae as described above, following Fan and Ho (1971). Parasitoid cocoons were collected in glass vials and kept in a clean Plexiglas cage (18 \times 15 \times 15 cm) until emergence. Adults were provided with a 20% honey–water solution. One-day-old, mated females, without oviposition experience, were used for experiments. Eggs of *C. carnea*, collected from a cabbage field, were placed individually in plastic cups (6 cm long \times 3 cm ID) and reared on DBM larvae as described above. Newly emerged adults were collected daily, fed with honey, fructose, protinex, water (1:1:1:1 and by volume), and transferred to acrylic cages (28 cm diam.) for oviposition. Three-6-day-old males and females were used for experiments.

Chemicals. Test compounds were obtained from commercial sources (Table 1). The synthetic pheromone blend was prepared by mixing Z11-16:Ald, Z11-16:Ac, and Z11-16:OH in 1:1:0.01 ratio. All solutions (1:100 v/v) were prepared in hexane.

Bioassays. Experiments were conducted in a Y-tube olfactometer made of transparent Plexiglas (3 cm ID; stem 10 cm, arms 8 cm; stem-arms angle 130°) with each arm connected to a glass container (250 ml) holding the odor source. Filtered air (30 ml/min for the parasitoids; 1.2 liters/min for the predator) was drawn through the olfactometer by a pump connected to a flowmeter. A vapor trail was generated with TiCl_4 to verify the airflow through the olfactometer. Whatman filter paper (4 \times 2 cm) impregnated with 10 μl of test solution, left to dry for 5 min, and introduced into one of the containers was used as the odor source. The other container held untreated filter paper of the same size. The olfactometer was disconnected from the containers and thoroughly washed in soap and water, rinsed in 70% ethanol, and dried in an oven at 120°C after every three runs. The apparatus was rotated 180° after five runs to exclude directional bias. Each insect was allowed

TABLE 1. PURITY AND SOURCE OF COMPOUNDS USED IN STUDY

Compound	Chemical purity ($\geq\%$)	Source
Hexane	99.5	Merck KGaA
Synthetic pheromone compounds		
Z11-16:Ald	97	Shin-Etsu Chemical Co. Ltd
Z11-16:Ac	97	Shin-Etsu Chemical Co. Ltd
Z11-16:OH	97	Shin-Etsu Chemical Co. Ltd
Larval frass volatiles		
Dipropyl disulfide	98	Aldrich Chemical Co. Ltd
Dimethyl disulfide	99	Aldrich Chemical Co. Ltd
Allyl isothiocyanate	95	Aldrich Chemical Co. Ltd
Dimethyl trisulfide	98	Kodak
Green leaf volatiles		
(Z)-3-hexenyl acetate	98	Sigma Chemical Co. Ltd
(E)-2-hexenal	98	Aldrich Chemical Co. Ltd
(Z)-3-hexen-1-ol	98	Merck KGaA
Hexanal	98	Merck KGaA
1-Hexanol	99	Sigma Chemical Co. Ltd
1-Hexen-3-ol	98	Aldrich Chemical Co. Ltd
Hexyl acetate	99	Aldrich Chemical Co. Ltd

to respond for 10 min and was used only once. Insect behavior was recorded with the program The Observer (Noldus Information Technology, Wageningen, The Netherlands). Responses were considered positive when insects traveled at least 4 cm along the arm connected to test compounds. The mean percentage of time spent by parasitoids/predator in each arm of the olfactometer was recorded and the values analyzed using the Wilcoxon matched-pairs test (Statistica, Stat Soft Inc.). All experiments were conducted in daylight at $28 \pm 2^\circ\text{C}$ and 60% relative humidity. To determine the optimum temperature for parasitoids/predator responses, tests were conducted at 15, 20, 25, 30, and 35°C using a blend of the DBM synthetic pheromone and (Z)-3-hexenyl acetate in 1:1 ratio.

RESULTS

Preliminary Y-olfactometer studies of *T. chilonis*, *C. plutellae*, and *C. carnea* indicated that hexane did not elicit any response from these insects. The parasitoids flew and/or walked towards the scent source, while the predator usually walked, hopped, or jumped since the olfactometer design did not allow them to fly.

Synthetic P. xylostella Pheromone. The responses of *T. chilonis*, *C. plutellae*, and *C. carnea* to pheromone blends and individual pheromone components are shown in Table 2. *T. chilonis* preferred the pheromone blend, the 1:1 blend of Z11-16:Ald and Z11-16:Ac, and Z11-16:Ac alone ($P \leq 0.001$) over the control,

TABLE 2. TIME SPENT IN EACH ARM OF Y OLFACTOMETER BY *Trichogramma chilonis*, *Cotesia plutellae*, and *Chrysoperla carnea* ADULTS WITH SEX PHEROMONE COMPONENTS OF DIAMONDBACK MOTH

Odor	Time spent in arm (%; mean \pm SD) ^a							
	Female <i>T. chilonis</i>		Female <i>C. plutellae</i>		Male		Female	
	Test	Control	Test	Control	Test	Control	Test	Control
Hexane	17.6 \pm 15.7	20.5 \pm 16.9	18.2 \pm 16.2	16.5 \pm 18.2	22.3 \pm 14.5	20.9 \pm 17.6	20.7 \pm 20.4	21.8 \pm 19.8
Pheromone blend ^b	46.0 \pm 28.3***	22.5 \pm 20.9	34.3 \pm 17.2**	17.4 \pm 20.7	35.2 \pm 21.7**	19.9 \pm 17.4	39.4 \pm 24.6***	17.0 \pm 15.4
Z11-16:Ald, Z11-16:Ac (1:1)	42.0 \pm 22.1***	19.7 \pm 17.0	36.3 \pm 20.4**	20.3 \pm 19.5	34.3 \pm 17.8*	22.6 \pm 20.7	35.2 \pm 22.3**	20.7 \pm 18.3
Z11-16:Ald	26.4 \pm 23.5	23.3 \pm 21.3	30.8 \pm 16.2*	18.3 \pm 16.8	19.2 \pm 17.3	16.5 \pm 15.4	21.4 \pm 18.6	20.2 \pm 17.1
Z11-16:Ac	48.0 \pm 31.8***	24.2 \pm 22.7	33.1 \pm 16.6*	21.8 \pm 19.7	21.5 \pm 18.6	22.7 \pm 20.7	17.5 \pm 16.2	19.5 \pm 17.9
Z11-16:OH	24.6 \pm 21.6	20.0 \pm 18.1	20.3 \pm 18.9	24.6 \pm 19.4	18.9 \pm 16.2	21.3 \pm 18.1	29.5 \pm 13.6	23.0 \pm 12.3

^a Asterisks indicate means significantly different from controls (Wilcoxon matched pair test, $N = 30$): * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

^b Pheromone blend was a mixture of Z11-16:Ald, Z11-16:Ac, and Z11-16:OH in 1:1:0.01 ratio.

while no preference was shown for Z11-16:Ald or Z11-16:OH alone. *C. plutellae* was attracted to the complete pheromone blend, the 1:1 blend of Z11-16:Ald and Z11-16:Ac, as well as the individual components other than Z11-16:OH, which had no effect. Similarly, *C. carnea* males and females preferentially responded to the ternary and binary pheromone blends, but not to the individual compounds.

P. xylostella Frass Volatiles. The responses of *T. chilonis*, *C. plutellae*, and *C. carnea* to DBM frass volatiles is presented in Table 3. *T. chilonis* females responded only to allyl isothiocyanate ($P \leq 0.01$), while the other volatile compounds failed to elicit significant responses. *C. plutellae* females responded to dipropyl disulfide ($P \leq 0.001$), whereas the other frass volatiles elicited responses at $P \leq 0.01$. *C. carnea* females showed preference for allyl isothiocyanate and dimethyl trisulfide ($P \leq 0.001$). These compounds also elicited smaller responses in males ($P \leq 0.01$ with allyl isothiocyanate, $P \leq 0.05$ with dimethyl trisulfide).

Cabbage GLVs. The response of *T. chilonis*, *C. plutellae*, and *C. carnea* to cabbage green leaf volatiles is shown in Table 4. Among the seven GLVs tested, only (Z)-3-hexenyl acetate elicited responses from the two parasitoids and the predator. In addition, *T. chilonis* was attracted by hexyl acetate ($P \leq 0.05$), and *C. plutellae* by (E)-2-hexenal and (Z)-3-hexen-1-ol ($P \leq 0.01$ and $P \leq 0.05$). When the pheromone was blended with the individual GLVs in a 1:1 ratio, the responses of *C. plutellae* and *C. carnea* were greater than those elicited by the pheromone or the single compounds alone.

Temperature. The responses of *T. chilonis*, *C. plutellae*, and *C. carnea* to blends of the pheromone and (Z)-3-hexenyl acetate in 1:1 ratio at different temperatures are presented in Table 5. For *T. chilonis* and *C. plutellae*, optimum responses were obtained at 30–35°C, although at 25°C the former also responded significantly. *C. carnea* males and females were mostly attracted at 20–25°C.

DISCUSSION

Among semiochemicals, sex pheromones play a prominent role in attracting parasitoids to the host (Nordlund et al., 1983; Noldus and van Lenteren, 1985; Colazza et al., 1997). In the present study, we demonstrate for the first time that DBM sex pheromone components, individually or as synthetic blends, are attractants for the parasitoids *T. chilonis* and *C. plutellae* and the predator *C. carnea*, three of the main natural enemies of the DBM worldwide.

Trichogramma spp. utilize a variety of semiochemicals including plant synomones and host kairomones to find their oviposition host (Nordlund et al., 1985). For instance, a synthetic blend of sex pheromone components of *Heliothis zea* (Z7-16:Ald, Z9-16:Ald and 16:Ald) increased rates of egg parasitization by *T. pretiosum* in greenhouse bioassays and field plots of cotton (Lewis et al., 1982).

TABLE 3. TIME SPENT IN EACH ARM OF Y OLFACTOMETER BY *Trichogramma chilonis*, *Cotesia plutellae*, AND *Chrysoperla carnea* ADULTS WITH FRASS VOLATILES OF DIAMONDBACK MOTH

Odor	Time spent in arm (% , mean \pm SD) ^a							
	Female <i>T. chilonis</i>		Female <i>C. plutellae</i>		Male		Female	
	Test	Control	Test	Control	Test	Control	Test	Control
Hexane	14.4 \pm 17.3	16.8 \pm 14.5	17.3 \pm 15.3	19.9 \pm 17.3	14.4 \pm 17.3	16.2 \pm 15.4	15.0 \pm 19.1	18.2 \pm 20.3
Dipropyl disulfide	22.6 \pm 22.4	18.5 \pm 16.2	38.0 \pm 22.1***	16.9 \pm 14.8	31.5 \pm 22.6*	19.7 \pm 18.0	35.4 \pm 22.6**	20.0 \pm 18.7
Dimethyl disulfide	25.3 \pm 21.1	20.4 \pm 18.3	36.5 \pm 18.5**	22.4 \pm 21.0	35.3 \pm 16.8**	20.4 \pm 23.7	30.7 \pm 21.3**	13.2 \pm 15.2
Allyl isothiocyanate	28.7 \pm 21.3**	15.2 \pm 14.4	34.5 \pm 17.3**	19.6 \pm 15.2	30.2 \pm 18.8**	14.7 \pm 16.9	37.0 \pm 22.1***	19.9 \pm 14.5
Dimethyl trisulfide	29.5 \pm 13.6	23.0 \pm 13.3	32.6 \pm 23.8**	16.1 \pm 15.4	36.5 \pm 18.9*	24.0 \pm 21.2	35.3 \pm 23.4***	17.7 \pm 15.9

^aAsterisks indicate means significantly different from controls (Wilcoxon matched pair test, $N = 30$): * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

TABLE 4. TIME SPENT IN EACH ARM OF Y OLFACTOMETER BY *Trichogramma chilonis*, *Cotesia plutellae*, AND *Chrysoperla carnea* ADULTS WITH GLVS OF CABBAGE (*Brassica oleracea* SUBSP. *capitata*), WITH OR WITHOUT DIAMONDBACK MOTH SEX PHEROMONE

Odor	Time spent in arm (%; mean \pm SD) ^a							
	Female <i>T. chilonis</i>		Female <i>C. plutellae</i>		Male		Female	
	Test	Control	Test	Control	Test	Control	Test	Control
Hexane	15.3 \pm 13.8	16.6 \pm 12.7	20.8 \pm 17.4	18.2 \pm 17.2	18.8 \pm 15.0	16.6 \pm 14.9	13.7 \pm 12.6	17.5 \pm 16.6
(Z)-3-Hexenyl acetate	41.0 \pm 20.7***	21.4 \pm 20.7	32.5 \pm 23.8**	16.5 \pm 18.0	31.8 \pm 22.4**	16.4 \pm 20.1	33.0 \pm 20.3**	17.5 \pm 15.2
(E)-2-Hexenal	16.8 \pm 13.6	19.4 \pm 15.8	30.0 \pm 21.4**	15.9 \pm 13.2	22.2 \pm 19.3	20.2 \pm 18.5	18.3 \pm 16.9	20.4 \pm 16.5
(Z)-3-Hexen-1-ol	21.0 \pm 18.4	19.6 \pm 16.3	30.8 \pm 21.4*	22.3 \pm 21.5	20.3 \pm 17.4	21.2 \pm 18.8	19.5 \pm 16.4	18.7 \pm 14.3
Hexanal	18.8 \pm 17.0	18.3 \pm 17.8	19.2 \pm 16.3	15.9 \pm 14.1	29.8 \pm 14.6	24.0 \pm 12.3	22.9 \pm 20.7	21.2 \pm 17.8
1-Hexanol	18.7 \pm 16.6	22.5 \pm 19.6	20.0 \pm 15.8	17.6 \pm 16.3	15.0 \pm 19.1	18.2 \pm 20.3	16.7 \pm 12.9	16.1 \pm 15.4
1-Hexen-3-ol	26.7 \pm 19.3	22.2 \pm 21.2	14.7 \pm 12.6	18.4 \pm 17.2	24.3 \pm 22.4	16.5 \pm 16.2	19.8 \pm 17.3	18.6 \pm 17.9
Hexyl acetate	32.3 \pm 19.4*	21.5 \pm 19.9	17.4 \pm 14.2	15.2 \pm 14.5	20.5 \pm 18.3	20.2 \pm 18.5	18.8 \pm 16.8	20.2 \pm 20.6
Synthetic pheromone (ph)	44.3 \pm 23.8**	22.8 \pm 18.4	33.6 \pm 18.6**	17.6 \pm 16.6	30.8 \pm 21.4*	22.3 \pm 21.5	38.5 \pm 23.4**	20.0 \pm 18.3
(Z)-3-Hexenyl acetate + ph ^b	48.4 \pm 26.2***	23.8 \pm 21.7	38.0 \pm 22.1***	19.9 \pm 14.5	45.2 \pm 24.3***	22.1 \pm 20.9	43.6 \pm 24.4***	21.3 \pm 19.1
(E)-2-Hexenal + ph ^b	41.3 \pm 22.7***	20.3 \pm 19.3	36.3 \pm 23.4***	20.7 \pm 16.0	34.3 \pm 20.7**	20.5 \pm 18.4	36.2 \pm 22.5**	24.8 \pm 21.4
(Z)-3-Hexen-1-ol + ph ^b	37.6 \pm 23.4**	21.8 \pm 17.4	30.6 \pm 20.3**	19.2 \pm 14.3	32.2 \pm 19.4**	19.4 \pm 16.9	34.3 \pm 21.8**	22.5 \pm 18.8
Hexanal + ph ^b	39.8 \pm 21.7**	19.0 \pm 17.3	31.1 \pm 21.8**	20.6 \pm 18.9	37.2 \pm 22.3**	22.8 \pm 19.0	30.3 \pm 22.7**	19.2 \pm 16.4
1-Hexanol + ph ^b	40.8 \pm 20.4**	21.5 \pm 20.7	33.7 \pm 18.6**	17.6 \pm 16.6	33.0 \pm 24.8**	21.4 \pm 18.8	35.6 \pm 24.8**	23.5 \pm 18.0
1-Hexen-3-ol + ph ^b	36.6 \pm 22.4**	20.0 \pm 18.3	34.2 \pm 19.3**	21.8 \pm 17.4	36.5 \pm 18.5**	23.4 \pm 21.9	32.5 \pm 23.7**	18.5 \pm 19.6
Hexyl acetate + ph ^b	40.2 \pm 20.7***	18.7 \pm 18.9	32.6 \pm 20.7**	20.4 \pm 18.6	33.8 \pm 24.9**	20.8 \pm 21.5	39.6 \pm 25.9**	23.2 \pm 21.9

^aAsterisks indicate means significantly different from controls (Wilcoxon matched pair test, $N = 30$): * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

^bIn 1:1 ratio.

TABLE 5. TIME SPENT IN EACH ARM OF Y OLFACTOMETER BY *Trichogramma chilonis*, *Cotesia plutellae*, AND *Chrysoperla carnea* ADULTS WITH BLEND OF SYNTHETIC DIAMONDBACK MOTH PHEROMONE AND (Z)-3-HEXENYL ACETATE IN 1:1 RATIO AT DIFFERENT TEMPERATURES

Temperature (°C)	Time spent in arm (% mean ± SD) ^a							
	Female <i>T. chilonis</i>		Female <i>C. plutellae</i>		Male		<i>C. carnea</i>	
	Test	Control	Test	Control	Test	Control	Test	Control
15	34.3 ± 22.8*	23.5 ± 22.8	30.8 ± 22.6*	21.4 ± 22.4	32.0 ± 18.5**	17.6 ± 15.8	31.6 ± 22.7**	19.5 ± 17.4
20	37.5 ± 23.5**	21.9 ± 20.3	32.5 ± 21.3*	22.7 ± 20.0	46.2 ± 22.7***	21.3 ± 23.4	48.4 ± 20.1***	24.3 ± 21.3
25	46.2 ± 25.5***	24.2 ± 23.0	33.5 ± 20.6**	17.4 ± 18.8	45.4 ± 23.8***	23.6 ± 20.1	46.8 ± 23.3***	25.6 ± 19.9
30	48.8 ± 26.2***	23.8 ± 22.7	36.7 ± 18.5***	20.9 ± 21.7	33.5 ± 20.2**	18.2 ± 16.2	34.0 ± 21.8**	17.2 ± 16.3
35	45.3 ± 22.4***	24.7 ± 20.6	36.4 ± 20.2***	19.6 ± 19.4	30.6 ± 18.3**	17.5 ± 18.8	31.3 ± 20.4**	20.5 ± 15.2

^aAsterisks indicate means significantly different from controls (Wilcoxon matched pair test, N = 30). *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.

These results were confirmed by Noldus (1988) in olfactometer bioassays. We have found that *T. chilonis* is attracted to the pheromone blend of the DBM and to one of the main pheromone components, Z11-16:Ac, alone or in combination with Z11-16:Ald, the other major component of the pheromone. Our results agree with those of Boo and Yang (2000), who reported that *T. chilonis* was attracted to the sex pheromone of *Helicoverpa assulta* and to a component of the pheromone blend Z11-16:Ac. The parasitoid was also attracted to E12-14:Ac, a component of the sex pheromone of *Ostrinia furnacalis* (Boo and Yang, 2000). However, contrasting results were obtained by Noldus and van Lenteren (1985), who reported that Z11-16:Ac is not always attractive to *Trichogramma* spp. Indeed, this chemical is also the main pheromone component of *Mamestra brassicae*, and elicited no responses in its parasitoid *T. evanescens*, in contrast to the response evoked by the sex pheromone released by calling female moths (Noldus and van Lenteren, 1985).

To our knowledge there are two reports on the response of *C. plutellae* to host plant volatiles. Bogahawatte and van Emden (1996) showed that female *C. plutellae* preferred the odor of the *Brassica* plant type on which they had developed, even over those volatiles from DBM-infested and damaged leaves of other *Brassica* plants. Potting et al. (1999) reported in wind-tunnel studies that this parasitoid uses stimuli from oilseed rape plants (*Brassica napus* cv. Falcon) in its in-flight searching behavior. An oviposition experience or contact with a host-damaged leaf prior to the bioassay significantly increased the response to these volatile cues. In our study, the DBM pheromone blend, as well as the two major components Z11-16:Ac and Z11-16:Ald, either individually or in a 1:1 blend, effectively attracted the braconid females.

We found that male and female *C. carnea* were also attracted to the DBM pheromone blend and to the pheromone blend devoid of Z11-16:OH. Similarly, two sex pheromone components of the pea aphid *Acyrtosiphon pisum* (Homoptera: Aphididae), (4a*S*,7*S*,7a*R*)-nepetalactone and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol, elicited significant electroantennogram (EAG) responses in both sexes of the lacewing (Zhu et al., 1999). However, female antennae were more sensitive to the lactone than to the lactol. In contrast, (*E*)- β -farnesene, a common component of the aphid alarm pheromone, elicited a 30% higher EAG response than either of the two former pheromone components, and (-)- β -caryophyllene, a potent inhibitor of the pheromone, also had an attractant effect on the predator (Flint et al., 1979).

Larval frass of DBM contains dipropyl disulfide, dimethyl disulfide, methyl propyl disulfide, dimethyl trisulfide, and allyl isothiocyanate, the three disulfides being responsible for the host searching behavior of the parasitoid *Diadromus pulchellus* (Auger et al., 1989). We tested these compounds, except the noncommercially available methyl propyl disulfide, and found that all chemicals exhibited responses from *C. plutellae* and both sexes of *C. carnea*, but not *T. chilonis*. *T. chilonis* was only attracted by allyl isothiocyanate. Notice the high attractivity

shown by the polyphagous predator *C. carnea*. This preference may be acquired during early developmental stages (Corbet, 1985), since *C. carnea* were reared on DBM larvae. We did not test predator populations reared on other hosts. Regarding frass activity on *C. plutellae*, only one previous report has been found on *Cotesia* spp: *C. marginiventris* attracted to hexane extracts of *Spodoptera frugiperda* frass and actual frass of the armyworm (Loke and Ashley, 1984).

We have previously identified several GLVs [(Z)-3-hexenyl acetate, (E)-2-hexenal, (Z)-3-hexen-1-ol, hexanal, 1-hexanol, 1-hexen-3-ol, and hexyl acetate] from cabbage (*B. oleracea* subs. *capitata*) (Reddy and Guerrero, 2000b). These chemicals induced attractant/arresting behavior in DBM in a wind tunnel and (Z)-3-hexenyl acetate enhanced the attractant effect of this synthetic pheromone blend in the field. When we tested these compounds in the olfactometer, they elicited strong responses in *T. chilonis*, *C. plutellae*, and both sexes of *C. carnea*, while (E)-2-hexenal and (Z)-3-hexenol also attracted *C. plutellae*. The attraction was greater when the GLVs were mixed with the pheromone blend in 1:1 ratio (Reddy and Guerrero, 2000b).

There are many reports on the effect of GLVs of cruciferous plants on insect pests (Visser et al., 1979; Guerin et al., 1983; Katsoyannos and Guerin, 1984; Zhang et al., 1999). However, there are few studies on the effect of GLVs on the natural enemies of these insect pests. Among these studies, volatiles emitted by sorghum (*Sorghum bicolor* L.) in the vegetative and reproductive stages arrested *T. chilonis* females, while volatiles from pigeonpea plant (*Cajanus cajan* L.) elicited no effect in the vegetative phase of the plant, and were repellent in the reproductive stage (Romeis et al., 1997). Extracts from corn leaves and the catnip *Nepeta catania* exhibited significant EAG responses in two predatory species, *Coleomegilla maculata* and *C. carnea* (Zhu et al., 1999). While many of the corn volatiles [(α -terpineol, (Z)-3-hexenol, (Z)-3-hexenal, 1-octen-3-ol, 1-hexanol, β -farnesene, 2-phenylethanol, and β -caryophyllene) attracted equal numbers of both sexes of *C. maculata* in the field, *C. carnea* adults were only allured by 2-phenylethanol (Zhu et al., 1999). In a previous but contrasting report, β -caryophyllene was found to act as an attractant of *C. carnea* in the field (Flint et al., 1979).

In the present study, the standard deviations are relatively large. This could be due to an effect of the experimental design, since the experiments were carried out at different times of the day and the responses may vary accordingly.

In summary, our results show that the parasitoids *T. chilonis* and *C. plutellae* and the predator *C. carnea* are attracted to a variety of chemical cues associated with their host, the DBM. These cues include sex pheromone components of the host, either in a blend or alone, frass volatiles, and GLVs from the host plant. The attraction of volatiles can be greatly enhanced when the GLVs are combined with the pheromone blend of the host. It is likely that the natural enemies considered utilize all these volatile compounds to locate the host.

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