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IDENTIFICATION OF OLFACTORY CUES USED IN HOST-PLANT FINDING BY DIAMONDBACK MOTH, *Plutella xylostella* (LEPIDOPTERA: PLUTELLIDAE)

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Abstract—Olfactory attraction of female diamondback moths (*Plutella xylostella*) to odors of intact and homogenized host plants, as well as individual compounds characteristic of host plants, were investigated by behavioral and electrophysiological methods. Moths were attracted to odors of *Brassica juncea* and *B. napus* seedlings in a Y-tube bioassay. Solvent fractions of homogenized *B. juncea* leaves were attractive to moths whether or not isothiocyanates (IC) were present. Moths were attracted in Y-tube bioassays and to field traps baited with individual ICs. Volatiles from *B. juncea* and *B. napus* elicited an electroantennogram (EAG) response and were attractive in the Y-tube bioassay. Allyl IC was shown to be the attractive component in homogenized plant volatiles but was found to be virtually absent from intact plant volatiles. Gas chromatographic fractionation of intact plant volatiles revealed a terpene-containing fraction to be most attractive to the moths. We were unable to isolate individual attractive compounds from this fraction. Our results suggest that certain elements of this fraction, possibly in combination, are important olfactory cues for host-plant finding by the diamondback moth with mustard oils playing an important and possibly synergistic role, particularly when plants are damaged.

Key Words—*Plutella xylostella*, Lepidoptera, Plutellidae, *Brassica*, host plant attraction, EAG, bioassay, host plant location, plant volatiles, mustard oils, isothiocyanates.

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INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.), is a highly mobile insect, of worldwide distribution, whose major host plants include most species of the Brassicaceae. In Canada, it is not capable of surviving the winters, but reinvades from the southern United States every spring (Smith and Sears, 1982). The adult must be capable of rapidly locating its host plants following migration or following local movement, for example, when the local host plants are unsuitable. As it is primarily a nocturnal insect, it is likely that odor cues are important in host-plant finding. This suggestion is supported by experimental results, which indicate that the diamondback moth responds to certain host-plant odors by moving upwind in their presence (Palaniswamy et al., 1986; Pivnick et al., 1990). Insects from at least five different orders are known to be attracted to volatiles of cruciferous plants (Feeny et al., 1970; Read et al., 1970; Wallbank and Wheatley, 1979; Shimizu and Usui, 1986; Pivnick et al., 1991). Where a primary attractant compound has been identified in crucifer-feeding specialists or their parasites, this has always been allyl isothiocyanate (allyl IC) (Feeny et al., 1970; Read et al., 1970; Free and Williams, 1978; Wallbank and Wheatley, 1979) and other isothiocyanates as well (Matsumoto, 1970). Isothiocyanates are hydrolytic breakdown products of glucosinolates, characteristic constituents of the Brassicaceae (Kjaer, 1960). In this paper, we investigate the response of diamondback moths to host-plant odors with the aid of a behavioral Y-tube bioassay, electroantennogram recordings, and field trapping.

METHODS AND MATERIALS

Insect Rearing

Insects were reared as previously described (Pivnick et al., 1990). Potted Oriental mustard *Brassica juncea* (L.) Czerniak (cv. Domo), grown in the greenhouse, was used for rearing and in all experiments.

Bioassays

Four different bioassays were used to assess or screen intact plants, plant extracts, and individual compounds, as follows:

Y-Tube Behavioral Bioassay. This bioassay has been described previously (Pivnick et al., 1990). Insects were tested on day 8 having been deprived of mates and plant odors for the previous 24 hr (where day 1 is 0–24 hr post-emergence). The bioassay lasted for the first 6 hr of the 8-hr scotophase. Tests were carried out with approximately 30 females per bioassay. Four bioassays were carried out per test for the initial concentration (500 mg plant equivalents) of fractions obtained in the solvent extraction and fractionation procedure

(described below). For lower concentrations, combined fractions, and all subsequent tests, five bioassays were carried out.

Electroanntennogram (EAG) Response. The signal detection and recording equipment and insect antennal preparation used for the EAG has been described previously (Chisholm et al., 1975). Odor samples were delivered to the insect antenna in 1-ml puffs. Test materials were administered through cartridges consisting of a glass pipet containing a small filter paper disk impregnated with the sample, with the exception of gas chromatograph (GC) fractions, which were tested directly from the GC collection tubes. The wide end of each cartridge was capped with a rubber septum. The cartridge was inserted into a glass delivery tube situated approximately 2 cm from the insect antennal preparation. Air was delivered through a needle tip inserted through the septum. Young females were found to respond best to plant compounds or extracts. Therefore, all EAGs were carried out on day 2 females (24–48 hr old), which had access to 10% sucrose solution until testing. The 1-ml air puffs were repeatedly administered in the following order: blank, standard, blank, test material, blank, standard, blank, and so on. Each moth was tested once with each test material, and a moth was used until a series was completed or until the moth's response to the standard diminished substantially. The standard was 1 μg phenylethyl IC in 10 μl of dichloromethane. Antennal responses to plant fractions are reported as a percentage of the mean response to the standard before and after the response to the test material. Mean response to the blank before and after was first subtracted from both the response to the standard and to the test material. Antennal responses to individual plant compounds are reported in millivolts.

Field Trapping. Baits used in field trapping were prepared so that release rates were relatively constant over the trapping period. Baits were placed in glass tubing, sealed at one end, with dimensions similar to those used by Pivnick et al. (1992). Tubing dimensions were identical for *n*-propyl IC and allyl IC. The release rate was directly proportional to the square of the diameter of the tubing and inversely proportional to the length of the air column above the test material. Release rates were measured during the experiments by measuring the length of the air column at the beginning and end of each trapping period. All traps were replicated four times.

Vertical cylinders of white cardboard (15 cm diameter and 30 cm tall) with Stickem Special (Michel & Pelton Company, Emeryville, California) on the outside served as traps. The traps, with baits placed in the center of the inside of the cylinder, were placed 10 m apart alongside plots of canola in Saskatoon for two periods of two weeks each, with baits replaced and bait loss measured after each two week period. The middles of the traps were placed on stakes at the height of the crop.

Traps were baited with compounds used in the EAG and Y-tube tests at release rates of approximately 4 and 0.4 mg/day. Allyl IC was also tested at 40

mg/day. Due to the small quantity of 3-methylthiopropyl IC available, it was not used in the field tests.

Oviposition and Larval Development. Ten pairs of newly emerged adult diamondback moths were placed in each of five cages containing one pot of one plant of 2-week-old *B. juncea* cv. Domo and one of a similar aged *S. alba* cv. Ochre plant plus a container of 10% sucrose solution. The moths were allowed to mate and oviposit for three days, at which time the adults were removed and the number of eggs on each plant counted. The plants were then maintained in separate cages while eggs hatched and larvae commenced feeding. When the majority of larvae had reached the second instar, 50 larvae from each cage were transferred to fresh, similar aged plants of the same species (10 larvae per plant) to complete development. Emergence of mature diamondback moths was then monitored.

Materials Tested

Intact Seedlings Tested in the Y-Tube. Oriental mustard, *Brassica juncea*; canola *B. napus* L. cv. Westar; white mustard, *Sinapis alba* L. cv. Ochre; and faba bean, *Vicia faba* L. cv. Outlook, were planted in 50 ml of sterile soil in 250-ml Erlenmeyer flasks. Twenty-five seeds of the first three species were planted and were tested seven days later when their total fresh weight (determined after the bioassay) was approximately 2 g. For *V. faba*, 10 seeds were planted and were tested after 10 days when the total weight was approximately 6 g.

Solvent Extraction and Fractionation. Leaves of 4-week-old *B. juncea* plants (25 g) grown in sterile growing media (Turface, Applied Industrial Material Corp., Deerfield, Illinois) in 15-cm-diameter pots, three plants per pot, were extracted with boiling methanol (100 ml) and 80% (v/v) methanol in water (Chisholm and Wetter, 1967). The methanol and some water were removed under reduced pressure at 50°C and the aqueous residue filtered and made up to 50 ml (fraction i). The aqueous solution of glucosinolates was sequentially extracted for 48 hr with pentane (fraction ii) and dichloromethane (fraction iii) to leave an aqueous extract (fraction iv). The latter was treated with myrosinase (EC .3.2.3.1) prepared from seeds of *S. alba* (Harris, 1970) and volatiles (fraction v) collected using a closed loop stripping apparatus as described previously (Pivnick et al., 1990). The myrosinase-treated solution was then extracted with dichloromethane as before (fraction vi) to leave an aqueous residue (fraction vii). The solvent extracts were concentrated to 0.5 ml by slow distillation through a 10-cm Vigreaux column at 50°C. Thus 10 μl solvent extract and 1 ml aqueous extract contained 500 mg plant equivalent (fresh weight). Samples were stored in the refrigerator until required. For Y-tube bioassays, 1 ml of aqueous extract was presented on a cotton wick or 10 μl of solvent extract was spotted on a 7-mm-diameter filter paper disk.

Individual Volatile Plant Compounds. Compounds were obtained from Aldrich Chemical Company, Milwaukee, Wisconsin (allyl IC = allyl isothiocyanate, 2-phenylethyl IC, *n*-propyl IC, 4-allylanisole, (1*S*)-(−)-verbenone, and α-terpineol), and the British Drug Houses, London, England (borneol). 3-Methylthiopropyl IC was synthesized according to published methods (Kjaer et al., 1955).

For both Y-tube and EAG bioassays, compounds were dissolved in dichloromethane at the appropriate concentration so that 10 μl spotted on 7-mm-diameter filter paper disks would contain the desired amount of compound. For EAGs, the disks were kept in the rubber septum-capped glass pipets and stored in a freezer when not in use. Each disk was used three times for EAG bioassays before discarding, during which time no change in EAG activity was noted. For the Y-tube bioassays, disks were used once only.

In EAG tests, all compounds were tested on five insects at three different doses: 0.1, 1, and 10 μg/disk. Compounds that elicited a significant response at the lowest dose were tested at two lower doses (0.01 and 0.001 μg/disk). Compounds were tested with the Y-tube bioassay with five replicates at five doses.

Collection of Volatiles and Gas Chromatograph Fractionation. Volatiles from 25 g of homogenized leaves of Oriental mustard were collected as described previously (Pivnick et al., 1990). After collection and elution, the resulting sample contained 500 mg fresh weight plant equivalents in 10 μl dichloromethane.

For collection of volatiles from intact seedlings, an open loop collection apparatus was constructed from a glass cylinder (21 cm OD × 45 cm high) fitted with an acrylic lid (25 cm OD × 2.5 cm thick) carrying three Swagelok fittings to accommodate inlet and outlet tubing (6 mm polyethylene) and a manometer. An O-ring was countersunk in the lid to make an air-tight seal with the rim of the cylinder. The lid was held in place by eye-bolts attached to a metal strip passed around the bottom of the apparatus. Traps were made by fusing two pieces of glass tubing 4 mm ID × 4 cm and 2 mm ID × 6 cm and placing 10 mg coconut charcoal (60 mesh, Fischer Scientific) between two plugs of polypropylene wool (Aldrich Chemical Company) in the wider section. Compressed air was passed through a Supelpure HC trap (Supelco Canada, Oakville, Ontario, Canada), and pressure was controlled using a pressure regulator and a relief valve made from the base of a Bunsen burner. Airflow through the apparatus was as follows: from the laboratory source air flowed through the Supelpure trap and pressure regulator into the cylinder via the inlet fitting, then out of the cylinder via the outlet fitting and through the charcoal trap. The apparatus was vented into the lab. With the design, airflows of 20–60 ml/min could be maintained while keeping the pressure increase inside the chamber to 100 Pa, or lower. Approximately 100 seedlings of *B. juncea* or *B. napus* cv. Westar were

grown in Turface in a 15-cm pot, which was placed in the chamber the day after seedling emergence (usually five to six days after planting) and volatiles were collected for 48 hr. Traps were kept at 50°C to minimize condensation of water and were eluted with 2 × 100 μl of dichloromethane. Of each 200 μl sample, 50 μl was taken and reduced to 10 μl under a nitrogen gas stream at 0°C.

Volatiles were separated with a Hewlett Packard model 5890A gas chromatograph equipped with a thermal conductivity detector (TCD) on a DB-5 column (30 m × 0.53 mm, d_f 1.0 μm, J & W Scientific Inc., Rancho Cordova, California) in a Hewlett Packard 5890A GC. The injector was kept at 200°C and the detector at 250°C. Helium (10 ml/min at 100°C) was used as carrier gas. A 5-μl splitless injection (250 mg plant equivalents) was made at 40°C and the temperature held for 4 min. The column was then heated to 225°C at 4°C/min, and held for 15 min. Timed fractions (Figure 1) were collected on 350-mm × 2-mm-OD glass tubes bent so as to fit into 400-ml Dewar flasks packed with crushed Dry Ice. The collection tubes were connected to the outlet of the TCD by short Teflon sleeves into which short glass plugs were inserted to seal the tubes after each fraction was collected.

For EAG bioassays, the glass collection tubes were fitted with short steel tubes by the Teflon sleeves. Air puffs were routed directly through the collection tubes at 0°C. Fractions were tested on one or two insects, and subfractions were tested on one insect only.

For Y-tube behavioral bioassays, the tubes were eluted with 40 μl of dichloromethane, and the eluate was transferred directly from the tube to a 7-mm filter paper disk. After the solvent evaporated, the test material was placed in one arm of the Y-tube for testing.

Compound Identification. Gas chromatograph–mass spectral analyses (GC-MS) were obtained with a Finnigan 4000 instrument carrying a DB-5 column (60 m × 0.32 mm., d_f 0.25 μm, J & W Scientific). The injector was kept at 200°C and the source at 300°C. Helium flowing at 50 cm/sec and 100°C was used as carrier gas. Compound identity was confirmed by comparison with retention times and mass spectra of authentic samples.

RESULTS

Moths responded positively to intact seedlings of *B. juncea* and *B. napus*, but not to *S. alba* or *V. faba*, (Table 1). Because of the lack of attraction to *S. alba*, it was decided to test its suitability as a host plant in comparison with *B. juncea*. The number of eggs laid on *B. juncea* (227 ± 37, $N = 7$) and *S. alba* (302 ± 40, $N = 7$) did not differ significantly ($t = 2.21$). The number surviving to adult emergence out of 50 second-instar larvae also did not differ significantly

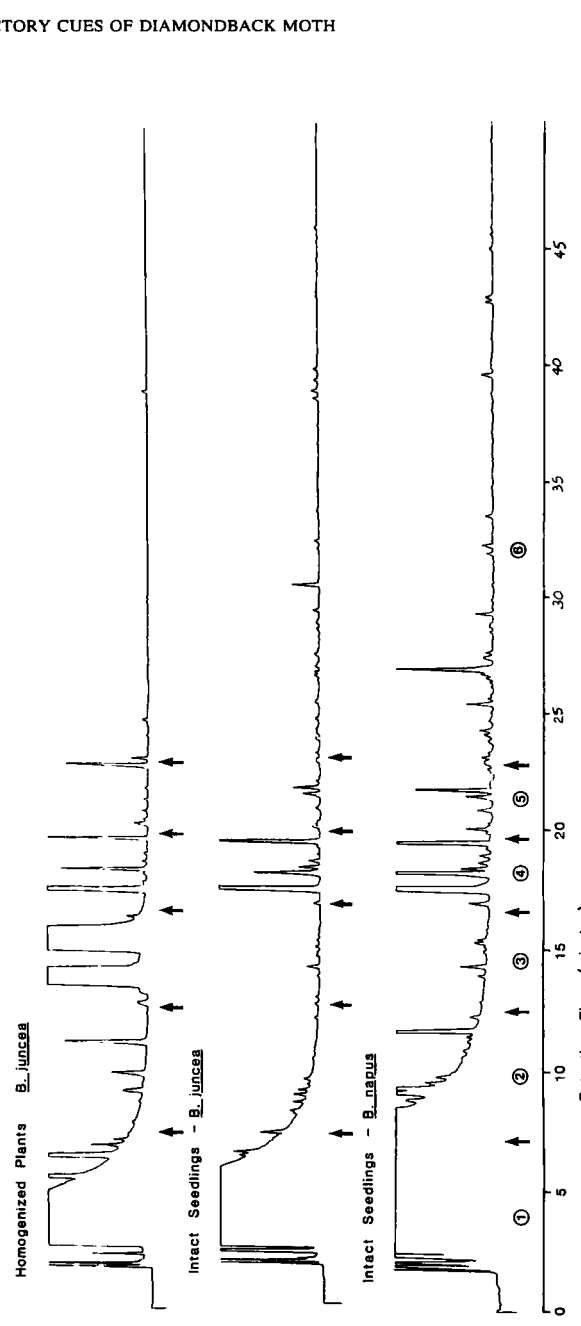

FIG. 1. Gas chromatograph (GC) traces of volatile collections from homogenized leaves of *Brassica juncea*; and intact seedlings of *B. juncea*, and *B. napus*. The arrows indicate the retention times at which cold-trapping collection tubes were changed in the first GC fractionation procedure to give fractions 1–6.

TABLE 1. Y-TUBE BIOASSAY RESPONSE OF *Plutella xylostella* FEMALES TO ODOR OF INTACT SEEDLINGS[a]

Species	Number of insects trapped		t^b	P^c
	Odor	Blank		
Brassica juncea	55	11	8.63	0.001
Brassica napus	60	14	4.89	0.01
Sinapis alba	39	25	2.12	NS
Vicia faba	19	26	3.09	NS

[a] Five replicates per test.
[b] Paired two-tailed t tests.
[c] NS, $P > 0.05$.

($t = 0.20$) between those reared on *B. juncea* (37.0 ± 5.5, $N = 5$) and those on *S. alba* (35.8 ± 4.4, $N = 5$).

Of the seven solvent fractions tested, all but fraction vi elicited a positive Y-tube response from the moths (Table 2). The crude aqueous fraction i remained attractive over a range of concentrations spanning four orders of magnitude. Fractions ii–iv, which ranged from nonpolar to polar and contained no isothiocyanates (verified by GC-MS), were all attractive. Fraction v, which contained volatile isothiocyanates (also verified), was attractive at only one dose (50 mg plant equivalents). Fraction vi, which was unattractive (at the one dose tested) also contained isothiocyanates (also verified). When fraction v at a subattractive dose was combined with fraction i at different doses, the response was consistently greater than that for fraction i alone at the same dose. However, the increase in attraction was small. When these two fractions were combined both at subattractive doses (when tested individually), the moths were attracted significantly, suggesting a weak additive or synergistic effect of two or more compounds in the mixture.

The lack of success in isolating attractive components with the solvent fractionation procedure prompted us to use GC fractionation of whole plant volatiles with EAG responses serving as an initial screening technique. These more rapid and finer fractionation and bioassay procedures were warranted as it was clear that a number of different attractive components were present. EAG tests on head space volatiles from homogenized *B. juncea* leaves showed that 250 mg fresh weight of plant equivalents was adequate for testing as female antennae responded strongly and consistently (Table 3). This material was even more stimulatory after collection from the gas chromatograph. The volatiles were separated into six fractions; the first five were approximately 5 min in

TABLE 2. Y-TUBE BIOASSAY RESPONSE OF *Plutella xylostella* FEMALES TO ODOR OF SOLVENT FRACTIONS OF *Brassica juncea* PLANTS AT FLOWER BUD STAGE

	Fraction	Concentration (mg plant equivalents)	Total number of insects trapped		t^a	P^b
			Odor	Blank		
(i)	Crude aqueous	500[c]	52	9	6.67	0.01
		50[d]	60	19	3.46	0.05
		5[d]	71	6	5.60	0.05
		0.5[d]	44	6	3.78	0.05
		0.05[d]	16	16	0	NS
(ii)	Pentane[c]	500	54	11	4.85	0.05
(iii)	Dichloromethane[c]	500	85	14	4.55	0.05
(iv)	Aqueous[c]	500	66	11	5.06	0.05
(v)	Volatiles after myrosinase treatment	500[c]	43	41	0.19	NS
		50[d]	75	4	6.96	0.01
		5[d]	35	20	0.73	NS
(vi)	Dichloromethane after myrosinase treatment[c]	500	69	30	1.19	NS
(vii)	Aqueous residue[c]	500	74	23	3.51	0.05
	Fraction (i) + (v)[d]	500 + 50	68	5	12.24	0.01
		50 + 5	86	6	11.35	0.01
		5 + 5	80	9	7.36	0.01
		0.05 + 5	22	9	2.99	0.05

[a] Paired two-tailed t tests.
[b] NS, $P > 0.05$.
[c] Four replicates per test.
[d] Five replicates per test.

duration, and the last, approximately 30 min (Figure 1; for exact times see Table 3). EAG activity was greatest for fractions 3 and 6, less so for fractions 4 and 5 and even less for fractions 1 and 2. Of all fractions, fraction 3 contained the greatest quantity of plant compounds and fraction 6 the least. Upon subdividing fractions 3 and 6, only subfraction 3.5 remained highly active (Table 3). This subfraction was the largest peak in the entire GC trace (other than the early solvent peaks) (Figure 1). Analysis by GC-MS and comparison with authentic samples established this component as allyl IC. The preceding large peak was similarly shown to comprise allyl thiocyanate and *trans*-2-hexenal, a "green plant volatile" (Visser and Avé, 1978). The allyl thiocyanate is likely to be an artifact of the GC procedure and not actually released by the plant (Slater, 1992).

TABLE 3. ELECTROANTENNOGRAM RESPONSE OF *Plutella xylostella* FEMALES TO HEADSPACE VOLATILES OF HOMOGENIZED *Brassica juncea* AND TO COLD-TRAPPED GAS CHROMATOGRAPH (GC) FRACTIONS OF VOLATILES

Test material	EAG response ($\bar{X} \pm$ SE)[a]	N	P[b]
Before GC injection			
250 mg plant equivalents	115 ± 17	3	0.05
50 mg plant equivalents	91 ± 31	3	NS
GC fractions (250 mg plant equivalents)			
Total GC effluent	277 ± 21	6	0.0001
Fraction 1 (8.00)[c]	32 ± 22	13	NS
Fraction 2 (13.00)	35 ± 13	9	0.05
Fraction 3 (17.80)	184 ± 30	9	0.001
Fraction 4 (21.30)	88 ± 20	9	0.01
Fraction 5 (24.75)	56 ± 10	9	0.001
Fraction 6 (50.25)	136 ± 21	9	0.001
Subfraction 3.1 (14.00)	41 ± 15	11	0.05
Subfraction 3.2 (14.50)	−1 ± 13	11	NS
Subfraction 3.3 (15.00)	57 ± 13	11	0.01
Subfraction 3.4 (15.85)	28 ± 10	11	0.02
Subfraction 3.5 (16.75)	173 ± 24	11	0.001
Subfraction 3.6 (17.80)	29 ± 10	11	0.02
Subfraction 6.1 (29.75)	56 ± 16	6	0.02
Subfraction 6.2 (34.75)	72 ± 30	6	NS
Subfraction 6.3 (40.00)	54 ± 16	6	0.05
Subfraction 6.4 (42.00)	56 ± 11	6	0.01
Subfraction 6.5 (50.25)	49 ± 15	6	0.05

[a] Responses reported are expressed as percent standard (1 μg phenylethyl isothiocyanate) administered before and after fraction.

[b] P is the probability that the mean EAG response is different from zero as determined by the t statistic; NS indicates that $P > 0.05$.

[c] Numbers in parentheses refer to the retention time (minutes) at the end of the fraction collection period.

All subfractions from fraction 6 elicited moderate EAG stimulation but no subfraction contained much activity, compared with fraction 6 in its entirety.

Y-tube bioassays (Table 4) of the headspace volatiles collected from homogenized *B. juncea* demonstrate that the moths readily discriminate between a solvent blank and the plant odor mixture, at higher and lower concentrations than that which elicited a strong EAG response (250 mg) (Table 3). The mixture was also attractive after 250 mg plant equivalents were injected and cold-trapped from the GC. Fraction 3 was attractive but all the other fractions combined were not. Subfraction 3.5 was attractive at 250 mg and at 25 mg but not at 2.5 mg plant equivalents. The remainder of fraction 3 was not attractive. A blank GC

TABLE 4. Y-TUBE BIOASSAY RESPONSE OF *Plutella xylostella* FEMALES TO HOMOGENIZED *Brassica juncea* HEAD SPACE VOLATILES AND TO COLD-TRAPPED GAS CHROMATOGRAPH (GC) FRACTIONS OF VOLATILES

Test material	Concentration (mg plant equivalents)	Number of insects trapped		t^a	P^b
		Odor	Blank		
Before GC injection	500	48	14	3.72	0.05
	50	49	4	9.00	0.001
GC fractions					
Fractions 1-6	250	63	10	3.01	0.05
Fraction 3	250	32	13	3.30	0.05
Fractions 1, 2, 4, 5, 6	250	21	8	0.22	NS
Subfraction 3.5	250	41	6	3.85	0.05
	25	34	4	4.47	0.05
	2.5	32	22	1.18	NS
Subfractions 3.1-3.4, 3.6	250	19	6	2.41	NS
Blank	0	20	17	0.19	NS

[a] Five replicates per test. Paired two-tailed *t* tests.
[b] NS, $P > 0.05$.

collection gave no response. We conclude that allyl IC is the main attractant in homogenized *B. juncea* volatiles.

Four isothiocyanates, which are known to occur in the Brassicaceae, were tested with the EAG. Of these, three elicited EAG responses at the two highest doses, while two compounds elicited significant EAG responses at the lowest level tested (0.001 μg) (Table 5). In Y-tube bioassay trials, allyl IC, 3-methylthiopropyl IC, and 2-phenylethyl IC were neutral at low doses, attractive at intermediate doses, and the latter two were repellent at the highest doses (Table 6). Traps containing the most attractive bait caught only twice the number of moths as did the blank traps during field trapping experiments (Table 7). For each compound, the higher the bait release rate, the higher the trap catch. With all compounds, significantly more moths were attracted at the highest release rate than with the blanks.

Allyl IC is primarily released when cells are ruptured, yet we have shown intact-plant odors also to be attractive to diamondback moths. Hence, we collected volatiles from intact seedlings, and repeated our GC fractionation procedure to isolate other attractive compounds. Volatiles from intact seedlings of *B. juncea* produced a strong EAG response when cold-trapped from the GC (Table 8). When the same timed fractions (as in Table 3; Figure 1) were assayed, the responses were similar to those elicited by the homogenized plant volatiles

TABLE 5. ELECTOANTENNOGRAM (EAG) RESPONSE OF *Plutella xylostella* TO FOUR INDIVIDUAL VOLATILE PLANT COMPOUNDS KNOWN TO OCCUR OR SIMILAR TO THOSE THAT OCCUR IN BRASSICACEAE

Compound[a]	Boiling point (°C)	EAG response, mV ($X \pm$ SE, $N = 5$), at different concentrations[b]				
		0.001 μg	0.01 μg	0.1 μg	1 μg	10 μg
Allyl IC	152			0.08 ± 0.07	0.42 ± 0.15*	0.48 ± 0.08**
n-Propyl IC	153			−0.13 ± 0.09	−0.09 ± 0.08	0.03 ± 0.14
3-Methylthiopropyl IC	240	0.32 ± 0.08*	0.57 ± 0.13*	0.68 ± 0.08*	1.03 ± 0.08***	1.36 ± 0.11***
2-Phenylethyl IC	280	0.18 ± 0.05*	0.27 ± 0.09*	0.74 ± 0.13*	0.84 ± 0.13**	1.16 ± 0.11***

[a]IC, isothiocyanate.
[b]$*P < 0.05$; $**P < 0.01$; $***P < 0.001$; probability that the mean EAG is different from zero as determined by the t statistic.

TABLE 6. Y-TUBE BIOASSAY RESPONSE OF *Plutella xylostella* TO INDIVIDUAL PLANT COMPOUNDS KNOWN TO OCCUR IN BRASSICACEAE

Compound (μg)[a]	Number of insects trapped		t^b	P^c
	Odor	Blank		
Allyl IC				
0.1	10	9	0.14	NS
1	26	8	3.88	0.05
10	59	9	18.26	0.001
100	57	10	23.50	0.001
1000	22	23	0.06	NS
3-Methylthiopropyl IC				
0.01	19	15	0.24	NS
0.1	54	10	3.96	0.05
1	84	7	14.95	0.001
10	20	30	2.25	NS
100	13	52	4.41	0.05
2-Phenylethyl IC				
0.1	25	18	0.50	NS
1	37	9	4.99	0.01
10	20	19	0.13	NS
100	8	51	2.66	NS
1000	0	28	9.30	0.001

[a] IC, isothiocyanate.
[b] Five replicates per test. Paired two-tailed t tests.
[c] NS, $P > 0.05$.

with one important exception. There was no significant response to fraction 3 (Table 8). All the other fractions elicited stronger EAG responses than in the previous fractionation procedure, although the relative strength of the responses was similar.

Fraction 6 (Fig. 1) was subdivided as before and all five subfractions elicited strong EAG responses (Table 8). The three most active subfractions (6.1, 6.2, and 6.3) were subdivided further and tested. Most of these subsubfractions were only moderately active but two (6.2.1, 6.3.2; Table 8) were very active. GC-MS analysis of subsubfraction 6.2.1 indicated at least 16 components (Figure 1, the middle GC trace) of which the following have been identified by comparing retention times (in seconds in parentheses) and mass spectra of available authentic compounds: the terpenes, borneol/isoborneol (1476), 4-terpinenol (1504), α-terpineol (1538), verbenone (1584), and *cis*-carveol (1606), as well as naphthalene (1516), estragole (4-allylanisole) (1554), and benzothiazole (1617). Peaks at retention times 1450, 1634, and 1665 sec appear to be silyl

TABLE 7. CAPTURES OF *Plutella xylostella* IN STICKY CYLINDRICAL TRAPS BAITED WITH PLANT COMPOUNDS KNOWN TO OCCUR IN BRASSICACEAE NEAR SASKATOON, JULY 20–AUGUST 17, 1989

Compound[a]	Release rate (mg/day)			Total number of moths[b]
	Estimated	Actual ($\bar{X} \pm$ SE)		
Allyl IC	40	55 ± 8	$N = 8$	328 a
2-Phenylethyl IC	4	9.6 ± 5.4	$N = 5$	313 ab
n-Propyl IC	4	2.8 ± 0.5	$N = 8$	256 abc
2-Phenylethyl IC	0.4	0.3 ± 0.1	$N = 8$	240 bcd
Allyl IC	4	1.2 ± 0.7	$N = 6$	225 cd
Allyl IC	0.4	0.3 ± 0.1	$N = 8$	225 cd
n-Propyl IC	0.4	0.3 ± 0.03	$N = 8$	210 cd
Control				157 d

[a]IC, isothiocyanate.
[b]Totals (of four replicates per bait) followed by different letters are significantly different at $P < 0.05$ as determined by two-way ANOVA and a protected LSD test.

compounds from pretreatment of the glass cylinder, while those at 1463 and 1497 seconds appear to be isomers with mass spectra similar to that of pinanone.

Volatiles from intact seedlings of *B. juncea* (Figure 1) were assayed in the Y-tube at three different doses. The intermediate dose (10% of a volatile collection) was most attractive and was, therefore, the main dosage used in the rest of the bioassays (Table 9). Cold-trapped volatiles collected from the GC were also active. Fraction 6 was attractive, but at a reduced dose (1%). Subsubfractions 6.2.1 and 6.3.2, which were highly active in the EAG (Table 8), were also attractive in the Y-tube bioassay, although in the latter case, the dosage of the material had to be increased to 100% of the GC collection for it to be attractive (Table 9). The four most abundant of the eight compounds identified by GC-MS analysis of subsubfraction 6.2.1 were tested in the Y-tube bioassay. The most abundant compound, α-terpineol, was tested at eight concentrations (0.001–10,000 μg), none of which were attractive to the moths. Verbenone, estragole, and borneol were also not attractive to the moths when tested at four concentrations (0.1–100 μg) in the Y-tube.

GC fractionation and EAG testing was repeated with volatiles collected from intact seedlings of *B. napus*. The EAG responses to these volatiles, fractions, and subfractions were very similar to those elicited by the corresponding volatiles, fractions, and subfractions of intact *B. juncea* seedlings (Table 8).

TABLE 8. ELECTROANTENNOGRAM (EAG) RESPONSE OF *Plutella xylostella* FEMALES TO VOLATILES FROM INTACT *Brassica juncea* AND *B. napus* SEEDLINGS FRACTIONATED BY COLD-TRAPPING ON GAS CHROMATOGRAPH (GC)

	B. juncea			*B. napus*		
GC fraction[a]	EAG response ($\bar{X} \pm$ SE)[b]	*N*	*P*[c]	EAG response ($\bar{X} \pm$ SE)[b]	*N*	*P*[c]
Fractions 1-6	341 ± 54	9	0.001	246 ± 35	10	0.001
Fraction 1 (8.00)[d]	61 ± 33	12	NS	−68 ± 13	10	0.001
Fraction 2 (13.00)	58 ± 15	12	0.01	9 ± 22	10	NS
Fraction 3 (17.80)	51 ± 24	12	NS	36 ± 26	10	NS
Fraction 4 (21.30)	103 ± 12	12	0.0001	126 ± 24	10	0.001
Fraction 5 (24.75)	130 ± 16	12	0.0001	139 ± 45	10	0.02
Fraction 6 (50.25)	454 ± 68	12	0.001	375 ± 47	10	0.001
Subfraction 6.1 (28.00)	271 ± 49	5	0.01	177 ± 37	5	0.02
Subfraction 6.2 (33.20)	334 ± 44	5	0.01	170 ± 41	5	0.02
Subfraction 6.3 (38.45)	255 ± 27	5	0.001	166 ± 31	5	0.01
Subfraction 6.4 (42.00)	120 ± 35	5	0.05	75 ± 22	5	0.05
Subfraction 6.5 (50.25)	152 ± 51	5	0.05	116 ± 9	5	0.0001
Subsubfraction 6.1.1 (23.30)	9 ± 15	5	NS			
Subsubfraction 6.1.2 (24.20)	7 ± 21	5	NS			
Subsubfraction 6.1.3 (24.75)	42 ± 18	5	NS			
Subsubfraction 6.1.4 (25.75)	39 ± 16	5	NS			
Subsubfraction 6.1.5 (26.85)	33 ± 17	5	NS			
Subsubfraction 6.1.6 (28.00)	61 ± 6	5	0.001			
Subsubfraction 6.2.1 (29.50)	149 ± 23	5	0.01			
Subsubfraction 6.2.2 (31.60)	47 ± 21	5	NS			
Subsubfraction 6.2.3 (33.20)	61 ± 30	5	NS			
Subsubfraction 6.3.1 (33.45)	4 ± 14	5	NS			
Subsubfraction 6.3.2 (37.90)	170 ± 34	5	0.01			
Subsubfraction 6.3.3 (38.45)	49 ± 20	5	NS			

[a] 25% of volatile collection was injected on the gas chromatograph.
[b] Responses reported are expressed as percent standard (1 μg phenylethyl isothiocyanate) administered before and after the fraction.
[c] *P* is the probability that the mean EAG response is different from zero as determined by the *t* statistic; NS indicates that $P > 0.05$.
[d] Numbers in parentheses refer to the retention time at the end of the fraction collection period.

DISCUSSION

It is not clear how diamondback moths actually respond to odor cues in nature. Based on what we do know about insect host-plant perception (see Metcalf, 1987; Visser, 1986) and location (see Finch and Skinner, 1982; Visser,

TABLE 9. Y-TUBE BIOASSAY RESPONSE OF *Plutella xylostella* FEMALES TO VOLATILES FROM INTACT *Brassica juncea* SEEDLINGS AND COLD-TRAPPED GAS CHROMATOGRAPH (GC) FRACTIONS OF VOLATILES

Test material	Percentage of collection used[a]	Number of insects trapped		N	t^b	P^c
		Odor	Blank			
Before GC injection	50	35	7	5	1.61	NS
	10	41	4	5	4.54	0.05
	1	38	10	5	2.39	NS
Fractions 1-6	10	69	18	5	7.97	0.01
	1	35	14	5	1.32	NS
Fraction 6	10	50	42	5	0.41	NS
	1	67	29	5	3.25	0.05
Fraction 6.2.1	10	79	17	5	3.71	0.05
	1	17	12	3	1.25	NS
Fraction 6.3.2	100	46	22	5	8.20	0.01
	10	12	18	3	1.00	NS

[a] Entire volatile collection injected on GC, cold-trapped and eluted. This is the percentage of eluate used in bioassays.
[b] Five replicates per test. Paired two-tailed t tests.
[c] NS, $P > 0.05$.

1988), diamondback moths probably fly low over vegetation in fields or weedy patches, and when favorable odors are detected from a short distance, they may move toward them, probably employing some combination of odor-conditioned anemotaxis, orthokinesis, and klinokinesis (see Kennedy, 1977), until they land. From this point, detection may be primarily due to detection of nonvolatile constituents including glucosinolates (Reed et al., 1989). In an agricultural setting, with vast monocultures of host plants, as exist in canola-growing areas of the Canadian prairies, host-plant location is likely to be simpler.

Diamondback moths respond positively to many different plant volatiles. Yet, results of our Y-tube bioassays with intact seedlings indicate specificity of response. The two *Brassica* spp. were strongly attractive. *S. alba*, a species that we demonstrate here to be an acceptable host, was not attractive. This same species contains oviposition-deterrent compounds, although these are overridden by oviposition stimulants present in significant amounts (Reed et al., 1989). Faba bean seedlings also were not attractive. In contrast, Palaniswamy et al. (1986) reported faba bean and *S. alba* extracts to be attractive to both sexes of diamondback moths. Our results are not strictly comparable to those of Palaniswamy et al. (1986) as we used intact seedlings, not homogenized leaf extracts, in our bioassay. Further, Palaniswamy et al. (1986) assumed independent assort-

ment of individual insects in their statistical analysis. We have found this assumption to be invalid and liable to lead to unfounded conclusions even when using insects of only one sex (unpublished data).

The results of our solvent fractionation of *B. juncea* suggest that there are other compounds as well as the mustard oils, both polar (soluble in water and dicholoromethane) and nonpolar (soluble in pentane), which attract diamondback moths. Moreover, allyl IC, present in large quantities when *B. juncea* is homogenized, was repellent to the moths at high concentrations. There was also some evidence of possible synergism between allyl IC and other *B. juncea* volatiles (see Table 2) as has been found with certain combinations of plant volatiles for other phytophagous insects (see Visser, 1986).

In our bioassays of GC-fractionated volatiles of homogenized *B. juncea*, which may be likened to volatiles produced by insect-damaged plants, the major stimulatory and attractive component was allyl IC. However, fraction 6 was also highly stimulatory in the EAG in spite of the small amount of volatile compounds indicated by GC (Figure 1). Since the Y-tube bioassays lasted 6 hr, it is perhaps not surprising that the small quantity of volatile material in fraction 6 was not attractive. While fraction 6 was found to contain minor amounts of 2-phenylethyl IC by GC-MS, the EAG activity was dispersed, indicating that several compounds are responsible for the collective EAG stimulation.

We were able to confirm our findings (regarding the importance of allyl IC) with EAG and Y-tube bioassays by obtaining pure compounds and testing them similarly. Allyl IC was found to be stimulatory with the EAG and attractive in the Y-tube bioassay over a definable range of concentrations. In field trapping experiments, allyl IC was attractive at the highest dose tested.

In addition to allyl IC, we tested *n*-propyl IC, 2-phenylethyl IC, and 3-methylthiopropyl IC. In the EAG, the latter two compounds elicited responses at a much lower dose than allyl IC, while *n*-propyl IC was not at all stimulatory. Clearly, the moths do perceive *n*-propyl IC as they were attracted to it in the field trapping experiment. While there is obviously some difference in response to allyl IC and *n*-propyl IC, the main difference between these two and the other two compounds in EAG response may result from their relative volatility (as reflected by their boiling points), since release rates from disks used in the EAG will depend on volatility. Extensive EAG testing of diamondback moths with other compounds, not reported here, leads us to conclude that the volatilities of allyl IC and *n*-propyl IC are close to the minimum boiling point that will elicit an EAG response at the maximum dose used (10 μg). Light et al. (1988) were able to obtain EAG responses to more volatile compounds such as ethanol. However, they used a larger filter paper disk for odor release and 10 times more compound, an amount identical to that used by Averill et al. (1988). In other studies of EAG responses to plant volatiles of which we are aware, methodology has been similar to ours (e.g., Ma and Visser, 1978), and volatiles tested with

EAG have generally been of molecular weight ≥ 99 (the molecular weight of allyl IC) (Metcalf, 1987). This inherent problem of sensitivity of the EAG to the combination of dose and volatility also indicates that some potentially active compounds will be missed when screening GC fractions with an EAG test.

Differences in volatility likely explain why allyl IC was not repellent at the highest dose tested in the Y-tube as were the other two compounds. Higher doses of allyl IC are likely to be repellent, as suggested when the allyl IC-dominated plant fractions were tested in the Y-tube. In field trapping, where volatility was not an issue because release rates were controlled, no important differences between compounds were noted. However, all compounds were weakly attractive at best. Even at a very high release rate, allyl IC attracted only twice as many moths as did the blank traps. This may indicate a short distance of response but odor competition undoubtedly affected these traps, which were on the edge of a canola field in late summer. Further attempts to field test other compounds including other isothiocyanates and terpenes proved fruitless due to low diamondback moth densities.

Isothiocyanates are probably the prime olfactory attractants to large patches of damaged plants. We have previously shown that *B. juncea* releases huge quantities of allyl IC over short time periods when damaged in a manner that simulates insect feeding (Pivnick and Jarvis, 1991). Pivnick et al. (1992) found that several isothiocyanates, including allyl IC and 3-methylthiopropyl IC, attract the flea beetles *Phyllotreta cruciferae* (Goeze) and *P. striolata* (F.), with allyl IC being the most attractive compound of those tested. In tests of volatiles of several homogenized crucifer species, the cabbage root fly, *Delia brassicae* (Wiedemann) is also attracted strongly to allyl IC (Wallbank and Wheatley, 1979). Allyl IC and *n*-propyl IC have also been shown to attract the northern false chinch bug, *Nysius niger* Baker, to field traps (Pivnick et al., 1991). However, in this case a synthetic mustard oil, ethyl-4-isothiocyanatobutyrate, proved to be more attractive than allyl IC. Based on these findings it would be easy to overestimate the role of allyl IC in attracting crucifer-feeding insects to their host plants. We have estimated that a group of 27,000 intact *B. juncea* at the flower bud stage would be required to produce enough allyl IC to attract nearby flea beetles or northern false chinch bugs (Pivnick and Jarvis, 1991). Thus, attraction of insects to isolated plants or small patches of plants in natural ecosystems almost certainly involves stimuli other than isothiocyanates alone, or is only effective from a few centimeters distant. For this reason, we investigated the response of diamondback moths to volatiles collected from intact host plants, where isothiocyanate concentration is much lower.

Tollsten and Bergstrom (1988) demonstrated that homogenized leaves of *Brassica* spp. release primarily five- and six-carbon alcohols, aldehydes, and acetates (the "green leaf volatiles" of Visser and Avé, 1978), as well as glucosinolate breakdown products (isothiocyanates, nitriles, and sulfides). On the

other hand, intact *Brassica* spp. release a diverse group of terpenes and some benzenoids, primarily benzaldehyde and phenylacetaldehyde, with very small amounts of the volatiles typical of homogenized leaves (Tollsten and Bergstrom, 1988). In light of this, our results indicate that diamondback moths are attracted to isothiocyanates when plants are damaged. While they may detect many other volatiles released from damaged plants, the quantities are less substantial and probably less important in attraction.

Tollsten and Bergstrom (1988) suggest that different plant stages may differ widely in the composition of volatiles released. The majority of the compounds present, however, should be the same. In our experiments, the decision to collect intact volatiles from seedlings rather than flower bud-stage plants, as we had done for homogenized plant volatiles, was based on the difficulty involved in collecting volatiles from large plants as compared to seedlings. We also felt that the plant stage difference would likely be dwarfed by the difference between intact and macerated plants.

Our EAG results indicate that the moths appear to be particularly sensitive to some combinations of plant volatiles, probably including terpenes, released from intact plants. These are the likely candidates for plant volatiles used as primary cues in host-planting finding when no damage is present. The presence of small amounts of isothiocyanates may increase responsiveness.

Our attempts to identify a primary attractant in intact crucifer volatiles have been unsuccessful to this point. It is interesting to note that one of the compounds identified in fraction 6, estragole, strongly attracts the western corn rootworm, *Diabrotica virgifera* Leconte (Lampman et al., 1987). It may be that the diamondback moth will not respond to a single compound in isolation (other than isothiocyanates), but rather responds to a mixture of odors more representative of the whole plant. This is suggested by the observation that when we subdivided fraction 6, the sub- and subsubfractions became less stimulatory in the EAG and correspondingly less attractive in the Y-tube bioassay. If there are two groups of primary cues dependent on damage level (e.g., terpenes or "green leaf volatiles" from undamaged plants and isothiocyanates from damaged plants), the level of damage necessary for a shift from predominance of one group to the other and the time course of the shift is not known.

In summary, diamondback moths detect and respond to a wide variety of compounds released by both intact and damaged plants. However, their high sensitivity to an as yet uncharacterized mixture, possibly dominated by terpenes in the volatiles of intact *Brassica* spp., would suggest that certain compounds from intact plants are of importance in host-plant finding. The strong response of diamondback moths to high levels of isothiocyanates typical of damaged plants indicates that these are likely also used in host-plant finding in certain situations. As well, low levels of isothiocyanates may strengthen response to mixtures of other plant volatiles.

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