

Behavioral Responses of the Diamondback Moth, *Plutella xylostella*, to Green Leaf Volatiles of *Brassica oleracea* Subsp. *capitata*

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Green leaf volatiles (GLVs) from *Brassica oleracea* subsp. *capitata* L. have been identified as 1-hexanol, (Z)-3-hexen-1-ol, 1-hexen-3-ol, hexanal, (E)-2-hexenal, hexyl acetate, and (Z)-3-hexenyl acetate, by their mass spectra and retention times in comparison with authentic samples. No isothiocyanates were found in the extract. The activity of these chemicals has been determined on mated and unmated males and females of the diamondback moth (DBM) *Plutella xylostella* in the laboratory (wind tunnel) and in the field. On unmated males, mixtures of (Z)-3-hexenyl acetate, (E)-2-hexenal, and (Z)-3-hexen-1-ol with the pheromone induced attractant/arresting behavior in 80–100% of the males tested, significantly higher than the effect induced by the pheromone alone. On mated males and unmated females the effect of the GLVs alone or in combination with the pheromone was poor, while on mated females these compounds elicited upwind flight and arresting behavior in 40–60% of the females assayed. There was no synergism when these chemicals were mixed with the pheromone. In the field, (Z)-3-hexenyl acetate, the most active GLV in laboratory tests, when mixed with the pheromone in 1:1 ratio, enhanced 6–7-fold the number of females and 20–30% the number of males caught by traps over those baited with the pheromone alone. Our results indicate that the enhancement of the attraction of both males and females of the DBM to traps baited with pheromone blended with the relatively inexpensive and environmentally safe (Z)-3-hexenyl acetate may be important for future control strategies of the pest.

Keywords: *Plutella xylostella*; Lepidoptera; Yponomeutidae; *Brassica oleracea* subsp. *capitata*; behavior; sex pheromone; green leaf volatiles; synergism

INTRODUCTION

Green leaf volatiles (GLVs) have been ubiquitously found in nature and characterized as saturated and monounsaturated short-chain aliphatic alcohols, aldehydes, and acetates (Visser et al., 1979). They result from the oxidative degradation of surface plant lipids and are produced when enzymes are liberated after plant tissue damage. Previous reports have shown that these compounds may act as host–plant attractants (Guerin et al., 1983; Katsoyannos and Guerin, 1984), fruit ripeness indicators (Engel et al., 1988), or defensive secretions (Hamilton et al., 1985). They also synergize the sex and aggregation pheromone effect on the bean and pea leaf weevil (Blight et al., 1984), boll weevil (Dickens, 1989), and the Mediterranean fruit fly and the smaller European bark beetle (Dickens et al., 1990). In Lepidoptera host–plant GLVs enhance the attractant activity of the sex pheromone of the corn earworm and the codling moth (Light et al., 1993). GLVs also reduce the attraction of the spruce bark beetle females to a synthetic pheromone when the GLVs were added to the source (Zhang et al., 1999). There are variable responses of the striped ambrosia beetle when GLVs are used with the aggregation pheromone in traps (Borden et al., 1997).

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) is a worldwide pest of

oil-seed and vegetable crops, particularly in cabbage and other cruciferous plants of the genus *Brassica*. The moth is present throughout the year in some parts of India and adjacent regions of Southeast Asia. It is capable of high mobility and long-distance migration, so adults may invade the original site. For instance it will invade Canada after overwintering in the southern U.S. each year (Smith and Sears, 1982).

The major components of the DBM pheromone are (Z)-11-hexadecenal (Z11–16:Ald) and (Z)-11-hexadecenyl acetate (Z11–16:Ac) (Tamaki et al., 1977; Chow et al., 1977). An 8:2 to 4:6 mixture of Z11–16:Ald and Z11–16:Ac is highly attractive to males in the field (Koshihara et al., 1978), but addition of only 1% of (Z)-11-hexadecenol (Z11–16:OH) to the bait significantly increased the capture of males (Koshihara and Yamada, 1980). Lure specificity is improved by adding 10% of (Z)-9-tetradecenol (Z9–14:OH) to the natural pheromone (Chisholm et al., 1983). The synthetic pheromone has been utilized in studies to monitor pest populations (Möttus et al., 1997; Reddy and Urs, 1995, 1996), in mass trapping experiments (Reddy and Urs, 1997), and in an IPM program (Reddy and Guerrero, 2000).

The DBM responds to a variety of plant odors, particularly those of *Brassica juncea* (*B. juncea*) and *B. napus* (Pivnick et al., 1994). The moths were attracted in Y-tube bioassays and to traps baited with isothiocyanates, the hydrolysis products of glucosinolates, which are characteristic constituents of the Brassicaceae. In this paper we report on the effect of GLVs on the behavior of males and females, mated and unmated,

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when each GLV was tested alone or in combination with the synthetic pheromone in the laboratory and in the field. The GLVs had been identified from *Brassica oleracea* subsp. *capitata* leaf extracts. Based on our studies, the GLVs can be efficient chemicals for improving the effectiveness of the synthetic pheromone in control strategies of this important pest.

MATERIALS AND METHODS

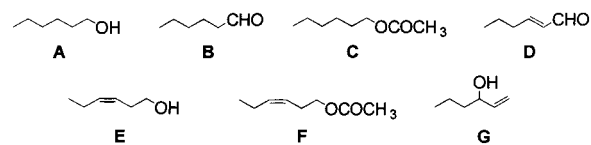
Insects. The diamondback moth, *Plutella xylostella*, was reared in the laboratory as previously described (Reddy and Urs, 1996). The cabbage *Brassica oleracea* subsp. *capitata* L., cv. Pride of India, was grown in a greenhouse under a 14:10 light:dark photoperiod at 30 ± 2 °C and 50% RH. Potted plants were used for rearing DBM. Newly emerged adults were provided with 10% sucrose solution and kept on filter paper in plastic containers. Handling of emerged adults was conducted as described by Chow and co-workers (Chow et al., 1984).

Plant Extraction and Chemical Analysis. Plant extracts were prepared by distilling fresh cabbage leaves of *Brassica oleracea* subsp. *capitata* L., according to the method of Craviers and co-workers (Craviers et al., 1989). Approximately 27 g of fresh leaves was heated in a 500 mL flask in a 700 W microwave oven for approximately 1 min. Volatile compounds were swept from the flask in a stream of nitrogen (flow rate, 60 mL/min) and trapped in a flask containing 50 mL of hexane, cooled at -10 °C. Several batches of volatiles were combined, dried (magnesium sulfate), filtered, concentrated to 5 mL, and diluted to prepare solutions of 1:100 (v/v) in hexane. The solutions were stored in the refrigerator until use. Analysis of the extracts was performed on a GC 6000 Vega Series 2 (Carlo Erba) coupled to a Finnigan Ion Trap 800 MS. A SGE HT8 25 m \times 0.25 μ m i.d. fused silica capillary column was used with helium as carrier gas. Mass spectra were obtained under electron impact (70 mV) conditions. Each GLV was identified by comparison of its mass spectrum and GC behavior with those of an authentic compound.

Chemicals. All chemicals (analytical-grade) were obtained from commercial sources. Hexanal (purity \geq 98%), (*E*)-2-hexenal (purity \geq 98%) and (*Z*)-3-hexenyl acetate (purity 98%), hexyl acetate (purity \geq 99%), (*Z*)-3-hexen-1-ol (purity 98%) and 1-hexen-3-ol (purity \geq 98%) were purchased from Aldrich/Sigma Chemical Co. Ltd, and hexane (purity \geq 99.5%) was purchased from Merck KGaA. Synthetic pheromone components were obtained from Pheromone Chemicals Ltd. (Tenali, India), and their purity was \geq 97%. For the pheromone blend Z11-16:Ald, Z11-16:Ac, and Z11-16:OH were mixed in a 1:1:0.01 ratio.

Wind Tunnel Bioassays. Bioassays were carried out in a wind tunnel, 240 cm long, 30 cm high, and 30 cm wide, as described by Singh and Majumder (1984). The tunnel was divided into four sections S1–S4, the first one (S1) being close to an electric fan which pulled air into the tunnel. The lengths of S1, S2, and S4 were 35 cm each, while S3 was 135 cm long. The dispenser with the attractant was hung in the center of S2, and the insects were released near the center of S4. The air speed in the tunnel was fixed at 40 cm/s, and an illumination of 5 lux was obtained from a dimmed fluorescent red light located at 40 cm above the tunnel. To facilitate experimentation during the working days, the moths were maintained under a reverse photoperiod (12:12 light:dark; onset of scotophase 12:00 a.m.). The test insects were introduced in the bioassay room at least 3 h prior the experiments to acclimatize them to the room conditions. A filter paper (4 \times 2 cm) was used as dispenser and suspended at a height of 15 cm. Each paper was impregnated with 10 μ L of the test compounds (either synthetic pheromone, GLV, or a mixture of both in 1:1 ratio) or solvent (hexane). For each replicate, two moths were released each time and their behaviors recorded for 10 min. Each treatment (chemical blend) was replicated 20 times (40 insects per treatment). The control treatment (hexane-treated) was replicated twice (four insects) on each day of experimenta-

Chart 1. List of the GLVs Found in a Leaf Extract of *Brassica oleracea* Subsp. *capitata*: A, 1-Hexanol; B, Hexanal; C, Hexyl Acetate; D, (*E*)-2-Hexenal; E, (*Z*)-3-Hexen-1-ol; F, (*Z*)-3-Hexenyl Acetate; G, 1-Hexen-3-ol



tion. One treatment (chemical blend) was completed each day, and then the tunnel was thoroughly washed before starting a new treatment. All treatments were carried out separately with mated and unmated males and females. The following types of behavior were recorded: wing fanning, circling (moths frequently walk around making one or more small circles), flying upwind, and landing at the source. The number of responding insects was analyzed using the χ^2 test (Sokal and Rohlf, 1981).

Field Trapping. Enhancement of the attractant activity of the synthetic sex pheromone by GLVs was assayed using (*Z*)-3-hexenyl acetate. Assays were completed in a cabbage field at the Agricultural Research Station (Hagari, Karnataka, India) from January to March and June to August 1997. A brown screw vial (23 mm long \times 5.5 mm wide \times 1.0 mm thick, Macherey-Nagel, Duren, Germany) fitted with a red rubber septum (Thomas Scientific, Swedesboro, NJ) onto the mouth was used as a dispenser. Each vial was filled with 1000 μ L of the test compound (either synthetic pheromone or (*Z*)-3-hexenyl acetate) and left at room temperature for 1 day before the experiment so that the rubber septum had been impregnated with the compounds. Delta sticky traps (18 \times 10 cm) were purchased from Pest Control Ltd. (Bombay, India) and baited with vials of pheromone, plant volatile, or both. The traps were randomly distributed in a 240 m² plot. Each trap was placed 30 cm above the crop canopy 1 week after transplantation (Reddy and Urs, 1996). The distance between traps was about 10 m. Traps and vials were rotated weekly and replaced every 15 days. Insects captured on the traps were removed weekly and identified, sexed, and cataloged. Six replicates (24 total traps) were used for each treatment. The temperature and wind speed during the experimentation period were recorded. The number of insects was subjected to a square root transformation ($\times+0.5$) and analyzed by one-way analysis of variance (ANOVA). The Tukey's test ($P < 0.05$) was used for separation of mean trap captures.

RESULTS

Analysis of Plant Extracts. GC-MS of the cabbage leaf extracts showed the presence of the following GLVs: 1-hexanol, (*Z*)-3-hexen-1-ol, 1-hexen-3-ol, hexanal, (*E*)-2-hexenal, hexyl acetate, and (*Z*)-3-hexenyl acetate (Chart 1). Surprisingly, no isothiocyanates were found in the extract.

Wind Tunnel Bioassays. Response of Unmated Males. In the wind tunnel unmated males responded positively to the synthetic pheromone (Table 1; $P \leq 0.01$). About 70% of males fanned their wings, 65% flew upwind toward the source, and 60% successfully landed. The GLVs (*Z*)-3-hexenyl acetate and (*E*)-2-hexenal elicited attractant behavior in 20–40% of unmated males. The number of insects contacting the source was 20–25% and was significantly higher than the control insects ($P < 0.05$). When blended with the pheromone, the most active GLVs were (*Z*)-3-hexenyl acetate, (*E*)-2-hexenal, and (*Z*)-3-hexen-1-ol, which induced attractant/arresting behavior in 80–100% of unmated males ($P < 0.001$, significantly higher (χ^2 test, $P \leq 0.05$) than those exposed to the pheromone alone. Particularly noteworthy is the activity displayed by the mixture (*Z*)-

Table 1. Percentage of Moths (Mated and Unmated) of *Plutella xylostella* Responding to Individual Plant Volatiles of *Brassica oleracea* Subsp. *capitata* and to Mixtures with the Synthetic Pheromone in a Wind Tunnel^a

compd	% of moths							
	unmated males		mated males		unmated females		mated females	
	flying	landing	flying	landing	flying	landing	flying	landing
hexane	5	0	0	0	5	0	5	0
synthetic pheromone (ph)	65**	60**	0	5	25**	15	5	0
(Z)-3-hexenyl acetate	40*	25*	30**	20*	20*	25**	60***	55***
(E)-2-hexenal	30*	20*	20*	15	20*	15	55***	50**
(Z)-3-hexen-1-ol	25*	15	0	10	10	15	40**	40**
hexanal	10	10	5	10	5	5	30*	25*
1-hexanol	15	5	0	5	5	5	15	20*
1-hexen-3-ol	20*	10	0	5	0	5	10	20*
hexyl acetate	10	5	0	5	5	0	5	10
(Z)-3-hexenyl acetate + ph	100***	95***	35**	35**	25**	30**	65***	60***
(E)-2-hexenal + ph	90***	85***	25**	25**	25**	20*	50**	50**
(Z)-3-hexen-1-ol + ph	85***	80***	15	20*	10	20*	40**	45**
hexanal + ph	80***	75**	5	10	10	10	35*	25*
1-hexanol + ph	75**	70**	5	10	5	5	25*	25*
1-hexen-3-ol + ph	65**	60**	5	10	0	5	35*	20*
hexyl acetate + ph	60**	55**	0	5	5	0	20*	15

^a Asterisks indicate differences (χ^2 test) from hexane: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

Table 2. Mean Catches (\pm SEM) Per Trap and Week of *P. xylostella* in Sticky Traps Baited with (Z)-3-Hexenyl Acetate, Synthetic Pheromone, and Mixture of Both in 1:1 Ratio in a Cabbage Field^a

chem blend	January–March		June–August	
	male	female	male	female
synthetic pheromone (ph)	910 \pm 21.3b	55 \pm 3.8c	1015 \pm 19.6b	63 \pm 3.2c
(Z)-3-hexenyl acetate	23 \pm 1.6c	114 \pm 18.5b	30 \pm 1.8c	126 \pm 5.2b
(Z)-3-hexenyl acetate + ph	1198 \pm 21.4a	385 \pm 17.4a	1216 \pm 22.8a	407 \pm 17.8a
control	13 \pm 1.2bc	2 \pm 0.2d	11 \pm 0.9c	6 \pm 0.6d

^a Means within the same column followed by different letters are significantly different ($P < 0.05$, Tukey's test).

3-hexenyl acetate and the pheromone, which induced practically all tested males to contact with the source.

Response of Mated Males. Mated males were not attracted to the pheromone blend, but 20–30% of them showed all four types of behaviors when (Z)-3-hexenyl acetate was used as the GLV source (Table 1). The other GLVs when tested alone were ineffective. Blends of (Z)-3-hexenyl acetate, (E)-2-hexenal, and pheromone had attractant activity only slightly higher than that induced by the GLVs alone. It should be noted that a higher number of males recorded landing at the source over the number of insects flying upwind can be explained by the fact that the DBM also walk to reach the attractant target.

Response of Unmated Females. The unmated females responded only slightly to their own sex pheromone and to most of the GLVs. The only exception was (Z)-3-hexenyl acetate which promoted flying and landing in 20–25% of individuals (Table 1). Mixtures of the GLVs with the pheromone did not significantly (χ^2 test, $P \geq 0.1$) affect virgin females over the effect induced on females by the GLVs alone.

Response of Mated Females. None of the mated females responded to synthetic pheromone. The GLV (Z)-3-hexenyl acetate evoked upwind flying and landing in 55–60% of females ($P < 0.001$). (E)-2-Hexenal elicited a similar effect on 50–55% of insects ($P < 0.01$), and (Z)-3-hexen-1-ol was active on 40% of females ($P < 0.01$; Table 1). Other GLVs were significantly active on fewer insects (20–25%, $P < 0.05$). There was no synergism when the GLVs were blended with the synthetic pheromone.

Field Trapping. A 1:1 mixture of (Z)-3-hexenyl acetate, the most active GLV found in the wind tunnel

bioassays, and the synthetic pheromone captured 20–30% more males than the synthetic pheromone alone. The plant volatile was ineffective on males when used alone in the trap. The GLV–pheromone blend induced also a 6–7-fold higher attraction on females than the synthetic pheromone alone. Moreover, the number of females caught was over 3-fold higher than that evoked by the GLV alone. The differences in all of the above cases were significant ($P < 0.05$, Tukey's test; Table 2). The average wind velocity and temperature were 4.2 m/s and 28.8 °C in January–March and 6.2 m/s and 37.4 °C in June–August.

DISCUSSION

GLVs are highly abundant in the plant kingdom and play an important role in plant–insect interactions (Visser, 1986). In *Brassica* spp. a number of GLVs have been reported (Wallbank and Wheatley, 1976; Cole, 1980a,b; Tollsten and Bergström, 1988; Fischer, 1992; McEwan and Smith, 1998), as well as glucosinolate breakdown products (isothiocyanates, nitriles, and sulfides) and benzenoids (Tollsten and Bergström, 1988). We extracted GLVs of *Brassica oleracea* subsp. *capitata* and identified seven compounds: 1-hexanol (A), hexanal (B), hexyl acetate (C), (E)-2-hexenal (D), (Z)-3-hexen-1-ol (E), (Z)-3-hexenyl acetate (F), and 1-hexen-3-ol (G) (Chart 1). Although we looked for the presence of isothiocyanates, there were no detectable isothiocyanates. Our results agree with those of Pivnick and co-workers (Pivnick et al., 1994) who found allyl isothiocyanate in homogenized leaf *B. juncea* volatiles but was lacking in intact plant leaves. This chemical also appeared to be present in macerated leaf samples of

Brassica carinata, *B. juncea*, and *B. nigra* but absent in *B. napus* and *B. campestris* (Tollsten and Bergström, 1988).

Commercial products have been routinely used to study the GLV effects on moths although they may contain impurities able to induce some behavioral consequences. In our case the effect of the impurities (carboxylic acids, alcohols, isomeric products, etc.) present was considered irrelevant in comparison to the major role played by the GLVs. These compounds induce variable types of electrophysiological and behavioral responses. Relatively strong electroantennogram (EAG) responses have been recorded on antennae of male and female *Ips typographus* when exposed to 1-hexanol, (*Z*)-3-hexen-1-ol, and (*E*)-2-hexen-1-ol (Zhang et al., 1999). Weak responses were elicited by (*E*)-3-hexen-1-ol and (*Z*)-2-hexen-1-ol, and no activity was caused by (*E*)-2-hexenal and (*Z*)-3-hexenyl acetate (Zhang et al., 1999). Low EAG responses on *M. brassicae* were recorded by Rojas (1999) except for 1-hexanol. In fact, 1-hexanol has been used as standard in EAG studies (Dickens, 1984). In our wind tunnel studies (*Z*)-3-hexenyl acetate, (*E*)-2-hexenal, and (*Z*)-3-hexen-1-ol significantly enhanced the responses of unmated males to the pheromone when mixed with the synthetic attractant in a 1:1 ratio. On mated males the synthetic pheromone was completely unattractive, and when the GLVs were mixed with the pheromone, the elicited effect was scarcely different to that shown by the GLVs alone. To our knowledge, this is the first report on the effect of GLVs on mated and unmated DBM males in a laboratory bioassay. In unmated females the effect of the GLVs was similar to that elicited on mated males and the synthetic pheromone evoked upwind flying on 25% of the insects. On mated females the effect of GLVs was more noteworthy. Again, (*Z*)-3-hexenyl acetate, (*E*)-2-hexenal, and (*Z*)-3-hexen-1-ol exerted all types of behavior on 40–55% of females, whereas other GLVs (hexanal, 1-hexanol, and 1-hexen-3-ol) induced also a smaller but significant attraction to the source. The synthetic pheromone was completely inactive, and mixtures of the GLVs with the pheromone did not practically enhance the effect produced by the individual GLVs. Our results are in contrast with those of Pivnick and co-workers (Pivnick et al., 1994), who suggested that the DBM does not respond to a single isolated compound but to a mixture of odors from the whole plant. Other studies have reported an increased attraction of females to blends of GLVs and pheromone, for instance, enhanced responses of Mediterranean fruit fly females to male odor in wind tunnel bioassays (Dickens et al., 1990). However, (*E*)-2-hexenal was completely inactive on *Acrolepiopsis assectella* females (Thibout et al., 1982), and (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, and 1-hexanol when added to the pheromone reduced female orientation to the source in *Ips typographus* (Zhang et al., 1999). Therefore, the activity of GLVs may be variable, inducing distinct effects on different insects.

We found that (*Z*)-3-hexenyl acetate was the most active GLV in the laboratory bioassay. In the field, the chemical significantly enhanced the number of females caught when mixed with the pheromone in a 1:1 ratio over those caught with the pheromone alone. The compound showed a slight but significant (χ^2 test, $P \leq 0.05$) synergistic effect in the capture of males. Alone, the chemical was attractive to females but not to males. Similarly, Light and co-workers (Light et al., 1993)

reported that pheromone traps containing (*Z*)-3-hexenyl acetate significantly increased the number of catches of *Heliothis zea* males over traps baited with the pheromone alone. (*E*)-2-hexen-1-ol enhanced trap catches of the boll weevil when blended with the pheromone, whereas hexanal and 1-hexanol exerted the same effect on the European elm bark beetle (Dickens et al., 1990). Other reports have shown a disruptive effect of GLVs. For instance, blends of (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, and (*Z*)-3-hexen-1-ol with lineatin, the aggregation pheromone of the striped ambrosia beetle, caused a 63–78% reduction in trap catches (Borden et al., 1997). Similarly, blends of 1-hexanol, (*Z*)-3-hexen-1-ol, and (*E*)-2-hexen-1-ol reduced *Ips typographus* catches by 85%, while ca. 70% trap reduction was achieved by blends of (*E*)-3-hexen-1-ol, (*Z*)-2-hexen-1-ol, and linalool (Zhang et al., 1999). Here in the field, as in the laboratory bioassays, the same GLVs may produce various effects in different insects, and this variability may be enhanced when the habitat of the insect falls within a wide geographic range (Borden et al., 1997).

It is unwise to generalize about the behavioral effects of GLVs and to attribute their effects only as host or nonhost volatiles. The activity shown by several GLVs on mated females, but not on virgin females, suggests that the GLVs attract mated females to the host plant for oviposition, as pointed out for allyl isothiocyanate in *M. brassicae* (Rojas, 1999). Moreover, some of the GLVs enhanced the pheromonal activity on unmated males both in the laboratory and in the field, suggesting that these chemicals are adequate olfactory stimuli to activate pheromone-mediated mate finding and arresting behavior on the DBM. We presume that the kairomonal basis for selection, assessment, and acceptance of a plant as an appropriate host may not only be part of the female's role but also, and perhaps to a lower extent, of that of the male. Enhancement of the insect pheromone action by GLVs could have important practical applications since insect pheromones cost is often high. Improvement of specific formulations by addition of a small amount of other minor components of the pheromone may be helpful, but it will also increase the manufacturer's cost. The use of inexpensive GLVs (between 0.1 and 5% of the cost of a typical pheromone component) could increase the effectiveness of the pheromone and diminish the amount of pheromone needed. In the case of the DBM the possibility of attracting not only males but also females to the traps by using (*Z*)-3-hexenyl acetate is an interesting additional feature to consider in future pest control studies.

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