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IMPACT OF BOTANICAL PESTICIDES DERIVED FROM *Melia azedarach* AND *Azadirachta indica* PLANTS ON THE EMISSION OF VOLATILES THAT ATTRACT PARASITOIDS OF THE DIAMONDBACK MOTH TO CABBAGE PLANTS

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Abstract—Herbivorous and carnivorous arthropods use chemical information from plants during foraging. Aqueous leaf extracts from the syringa tree *Melia azedarach* and commercial formulations from the neem tree *Azadirachta indica*, Neemix 4.5®, were investigated for their impact on the flight response of two parasitoids, *Cotesia plutellae* and *Diadromus collaris*. *Cotesia plutellae* was attracted only to *Plutella xylostella*-infested cabbage plants in a wind tunnel after an oviposition experience. Female *C. plutellae* did not distinguish between *P. xylostella*-infested cabbage plants treated with neem and control *P. xylostella*-infested plants. However, females preferred infested cabbage plants that had been treated with syringa extract to control infested plants. Syringa extract on filter paper did not attract *C. plutellae*. This suggests that an interaction between the plant and the syringa extract enhances parasitoid attraction. *Diadromus collaris* was not attracted to cabbage plants in a wind tunnel and did not distinguish between caterpillar-damaged and undamaged cabbage plants. Headspace analysis revealed 49 compounds in both control cabbage plants and cabbage plants that had been treated with the syringa extract. Among these are alcohols, aldehydes, ketones, esters,

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terpenoids, sulfides, and an isothiocyanate. Cabbage plants that had been treated with the syringa extract emitted larger quantities of volatiles, and these increased quantities were not derived from the syringa extract. Therefore, the syringa extract seemed to induce the emission of cabbage volatiles. To our knowledge, this is the first example of a plant extract inducing the emission of plant volatiles in another plant. This interesting phenomenon likely explains the preference of *C. plutellae* parasitoids for cabbage plants that have been treated with syringa extracts.

Key Words—Botanical pesticides, parasitoid behavior, *Plutella xylostella*, induced plant volatiles, elicitor.

INTRODUCTION

The diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is a major pest of crucifer crops and is found throughout the world (Talekar and Shelton, 1993). Natural enemies are recognized as important components in *P. xylostella* management strategies, particularly where control with chemicals has failed. However, they need to be integrated with other strategies for successful control. Botanical pesticides are thought to be compatible with biological control, as they tend to be relatively harmless to parasitoids and predators (Schmutterer, 1995, 1997; Charleston et al., 2005). In South Africa, 21 species of parasitoids are associated with *P. xylostella* (Kfir, 2003), providing a rich and abundant source for biological control. Botanical pesticides have not been registered for use in South Africa. However, the syringa tree *Melia azedarach* L. (Meliaceae) is an invasive plant found throughout South Africa. It has insecticidal properties (Ascher et al., 1995) and may provide the small-scale rural farmer with an alternative control tactic. It is important to investigate the impact that extracts have on the natural enemy fauna. Botanical pesticides act as repellents against a number of pest species, but little information is available concerning their effect on the behavioral responses of natural enemies of pests such as parasitoids and predators (Akol et al., 2003).

Plants influence carnivore behavior (Dicke et al., 1990; Turlings et al., 1990; Steinberg et al., 1993; Dicke and Vet, 1999; Vet, 1999; Dicke, 1999a; Hilker and Meiners, 2002). Plants that are attacked by herbivores emit volatile cues that can be used by natural enemies of the herbivore to find their hosts, and these plant volatiles may contain information on the identity of the herbivore (Turlings et al., 1990, 1993; Vet et al., 1991; Vet and Dicke, 1992; Dicke and Vet, 1999). The main components of the volatile blend released from cabbage plants are terpenoids and green leaf volatiles (Mattiacci et al., 1994; Shiojiri et al., 2001). Terpenoids are a major class among herbivore-induced synomones that attract carnivores (reviewed by Takabayashi et al., 1994; Dicke, 1994;

Turlings et al., 1995; Pichersky and Gershenzon, 2002). Terpenoids are released in analogous amounts in both herbivore-damaged and mechanically-damaged cabbage plants, as well as in undamaged plants (Mattiacci et al., 1994; Shiojiri et al., 2001). Plants emit green leaf volatiles during aging or when injury occurs (Visser and Ave, 1978; Hatanaka, 1993). However, there is a dramatic increase of green leaf volatiles in the headspace of damaged cabbage plants compared to undamaged plants, and this may play a role in parasitoid attraction (Mattiacci et al., 1994; Shiojiri et al., 2001; Smid et al., 2002). For example, *Cotesia rubecula* and *Cotesia glomerata* (Hymenoptera: Braconidae) can distinguish between damaged and undamaged Brussels sprout plants (Steinberg et al., 1992; Geervliet et al., 1996). Artificially damaged cabbage plants also produce volatiles, but these do not show any qualitative differences compared to the volatiles released when the plant is damaged by herbivores (Mattiacci et al., 1994; Geervliet et al., 1997; Shiojiri et al., 2001). However, there are quantitative differences (Mattiacci et al., 1994; Shiojiri et al., 2001). *Cotesia glomerata* did not distinguish between artificially damaged cabbage plants and plants damaged by its host *Pieris rapae* (L.) (Lepidoptera: Pieridae) (Shiojiri et al., 2001). However, *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) distinguished between artificially damaged cabbage plants and plants damaged by *Plutella xylostella* (Shiojiri et al., 2001), and the parasitoid spent longer searching infested cabbage plants than artificially damaged plants (Shiojiri et al., 2000a). Chemical analysis of the volatiles released by cabbage plants that had been damaged by two different host species, *P. xylostella* and *Pieris rapae*, indicated that there were slight qualitative differences (Agelopoulos and Keller, 1994) and many quantitative differences in the compounds produced in response to damage from these two herbivores (Agelopoulos and Keller, 1994; Geervliet, 1997; Shiojiri et al., 2001). *Cotesia plutellae* discriminated between cabbage plants infested with *P. xylostella* (host) and plants infested with *Pieris rapae* (nonhost) (Shiojiri et al., 2000b, 2001). The searching time of *C. plutellae* on cabbage leaves infested by host larvae was also longer than on leaves infested with the nonhost (Shiojiri et al., 2000a), which suggests that this parasitoid can discriminate between host- and nonhost-infested plants through antennal contact (Shiojiri et al., 2001, 2000a).

Cotesia plutellae and *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae) are the two parasitoid species most commonly found in cabbage fields around Pretoria, South Africa. We have shown that aqueous leaf extracts from the syringa tree *Mella azedarach* and the neem product, Neemix 4.5®, taken from the neem tree *Azadirachta indica* Juss. (Meliaceae) do not have a direct impact on the survival of these parasitoids (Charleston et al., 2005), and that they are still able to find their hosts when host-infested plants have been treated with the botanical extracts. In fact, in both greenhouse and field experiments, *C. plutellae* parasitize a greater proportion of *Plutella xylostella*

larvae on cabbage plants treated with syringa extracts than they do on control plants (Charleston et al., 2005). Small differences in plant volatiles can affect the attraction of parasitoids, and treating plants with botanical extracts may change the volatile profiles, which may have an impact on the responses by natural enemies. Here, we investigated the impact that these botanical extracts have on the volatile profile of cabbage plants and how this may influence the attraction of *C. plutellae* and *D. collaris*.

METHODS AND MATERIALS

Experimental Plants and Insects. Cabbage plants *Brassica oleracea* var. *capitata* L. (Cruciferae) were bought as seedlings, planted in black plastic bags, and left in a glasshouse (30 ± 5°C) to grow. The plants were fertilized with compost when planted and regularly watered. To reduce insect damage, the plants were placed inside a tent-like construction composed of fine nylon netting (mesh size <1 mm).

Plutella xylostella were from a culture originally collected near Pretoria (28°15′S; 25°44′E) and Brits (25°38′S; 27°47′E), South Africa. The laboratory culture was maintained on canola seedlings *Brassica napus* L. (Cruciferae). The two parasitoid species most common in cabbage fields near Pretoria, South Africa, were *Cotesia plutellae* and *Diadromus collaris*. Laboratory cultures of these were established in 1993. Each parasitoid species was kept communally in glass cages (38 × 27 × 28 cm) and exposed to *P. xylostella* larvae on canola seedlings three times per week. After exposure to *C. plutellae*, the second instar *P. xylostella* larvae were maintained on cabbage leaves in shallow plastic rearing containers until cocoon formation. To obtain fresh pupae for *D. collaris*, they were exposed to late fourth instar *P. xylostella*, which would pupate within 1 d. Cocoons of *C. plutellae* or *P. xylostella* pupae parasitized with *D. collaris* were placed into clean cages, and the emergence of wasps took place in clean cages without any plant or host material. The parasitoids were maintained on a diet of honey and water. Parasitoids used in the bioassays were 2–6 day old mated females.

All insect rearing was carried out in a controlled environment (24 ± 2°C; 65 ± 5% RH, 16:8 hr light/dark period).

Botanical Extracts. Syringa: Melia azedarach (hereafter referred to as syringa) leaves were collected from Rietondale in Pretoria, South Africa (28°15′S, 25°44′E). Leaves were collected from trees at a height of about 1.5–3.5 m at the start of spring flush, in September 2002, placed in a glasshouse (30 ± 5°C) to dry, after which they were crushed into a fine powder and stored in an airtight container until use. The extract was made with 100 ml of distilled water. The water was heated to 48°C; 5 g of leaf powder were added to the water, and the mixture was shaken for approximately 1 min. The extract was left in a refrig-

erator (±4°C) overnight. The following morning, it was filtered using Advantec® filter paper no. 2. Three drops of liquid detergent (Teepol®) were added to the final extract to act as a surfactant, without which the extract runs off the surface of the leaf.

Neem: A commercial preparation of *Azadirachta indica*, Neemix 4.5® (hereafter referred to as neem), was provided by Thermo Trilogy Corporation, Columbia, MD, USA. A dose of 32 µl per 100 ml of distilled water was used. Three drops of liquid detergent were added to the final solution.

Control: The control treatment used consisted of 100 ml of distilled water mixed with three drops of liquid detergent.

Wind Tunnel Design. A wind tunnel was set up within a tent-like construction (350 × 300 × 200 cm). It was placed in a room with a controlled temperature of 24 ± 2°C. No daylight could enter the room. Illumination was provided by rows of lights in the roof of the tent, simulating daylight (2300 lx). Two table fans were placed at the end of a table; a sheet of gauze material (mesh size, 3 mm) was placed in front of the fans to reduce the wind speed and to provide a more laminar airflow. The wind speed at the release point was approximately 0.133 m/sec. The cabbage plants were placed on a table (180 × 900 cm; Figure 1). The table was covered with white plastic sheeting to facilitate cleaning, and thick (4.7 cm) black strips of tape were placed at 30-cm intervals to provide a contrast for the flying insects. To contain the insects within the experimental arena and to create diffuse lighting, white cotton sheeting was placed across two poles 112 cm above the table and left to hang over the sides, enclosing the arena. An equilateral triangle (45 cm) was marked out on the table, and cabbage plants of the same age (±5 wk after transplant) were placed in groups of four on either side of the base of the triangle. The plants were placed in a square around the mark, with 20 cm between the centers of each plant. One group of plants was treated with the botanical pesticide and the other with control solution. The plants were changed to opposite sides of the triangle after every five observations.

Behavioral Recordings. Parasitoids were released from a platform at a 20-cm height, at the apex of the equilateral triangle at the downwind end of the tunnel (Figure 1). A female parasitoid was faced with a choice between treated and control cabbage plants and was observed to assess which plant she landed on first. "Response'' was recorded when the female left the release platform and landed on one of the plants. For each treatment, 60 responding females were observed. The number of females that did not respond was also recorded. "No response'' occurred when the female failed to leave the platform after 5 min, or if the female landed on any surface other than a cabbage plant.

Choices between treated and control plants were analyzed by using binomial probability functions to assess a difference from a 50–50 distribution between the treatment and control.

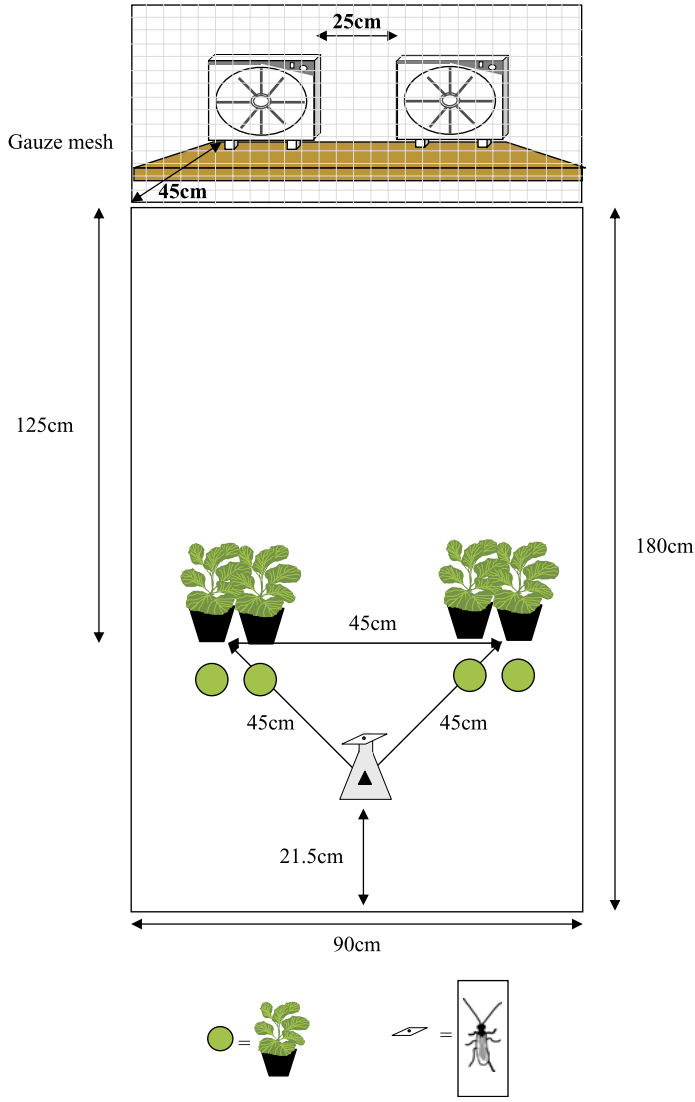

FIG. 1. Diagram showing the design of the wind tunnel.

Previous Experience. We observed that *Cotesia plutellae* did not respond in the wind tunnel without an oviposition experience. Potting et al. (1999) have shown that an oviposition experience significantly increased the response of *C. plutellae* to volatiles. Therefore, each *C. plutellae* female was exposed to a

cabbage leaf with feeding *Plutella xylostella* larvae. Each female was allowed to oviposit two to three times before being removed and placed into a glass vial with some honey for food. Approximately 1–2 hr later, she was released in the wind tunnel and observed.

Diadromus collaris females were also given an experience before they were released in the wind tunnel. In this case, the female was exposed to a cabbage leaf that had been damaged by *Plutella xylostella* larvae, which had subsequently pupated on the leaf just before exposure to *D. collaris*. The female was left to explore the leaf for 3 min, after which she was removed and placed in a glass vial with some honey for food, and released in the wind tunnel 24 hr later.

Experiments

Plant–Host Complex. Cabbage plants were first sprayed with either the botanical pesticide or the control (approximately 100 ml per plant) and then infested with 15 *Plutella xylostella* larvae (second instar for test with *Cotesia plutellae*, or late fourth instar for test with *Diadromus collaris*). Larvae were left to feed on the cabbage plants for 24 hr, after which infested plants were placed in the wind tunnel and the observations began. To compensate for any differences between the cabbage plants themselves, the experiment was carried out over three different days using different plants and different wasps each day.

Effect of Experience. This trial was also used to investigate whether previous experience on cabbage treated with the plant extracts had an influence on the subsequent flight behavior of the parasitoid in the wind tunnel. Each species was given experience with *Plutella xylostella* larvae (for *Cotesia plutellae*) or pupae (for *Diadromus collaris*) on either treated or untreated cabbage leaves, and the subsequent behavior was compared. Data were analyzed with a chi-square test.

Response of Pupal Parasitoid. Because *Diadromus collaris* showed a low responsiveness in the wind tunnel (<50% responded), we carried out an additional experiment to investigate whether this species responded to volatiles released from damaged plants. For this experiment, one group of cabbage plants was undamaged, and one group was damaged by 15 late fourth instar *Plutella xylostella* larvae, which were left to feed and pupate. Approximately 48 hr later, pupae were removed from the cabbage plants, and these plants were exposed to *D. collaris* in the wind tunnel, with undamaged plants for comparison. The groups of plants were replaced three times during the experiment. Again, less than 50% of the *D. collaris* females responded to the plants and, therefore, no further experiments were carried out with this species.

Different Syringa Doses. Further experiments were carried out with *Cotesia plutellae* using two lower doses of the syringa extract. The lower doses

were made with 1 and 3 g of leaf powder and 100 ml of distilled water, and prepared as described above (Plant–Host Complex).

Damaged Plants without Hosts. To investigate whether the presence of the host itself resulted in an attraction of *Cotesia plutellae*, the experiment was repeated as described above (Plant–Host Complex). However, *Plutella xylostella* larvae were allowed to feed for 24 hr and then removed from the plant before the plants were exposed to *C. plutellae* in the wind tunnel. Only the host larvae were removed; their by-products (frass, silk, etc.) remained on the plant.

Equally Damaged Plants without Hosts. Schuler et al. (1999, 2003) have shown that the amount of damage is an important factor influencing the response of *Cotesia plutellae*. Because botanical extracts reduce the feeding of *Plutella xylostella* larvae (Charleston, 2004), the experiment was repeated to compensate for any possible differences in the amount of damage. To create cabbage plants that had an approximately equal amount of damage, plants were first infested with 15 second instar *P. xylostella*. The larvae were left to feed on the plants for 24 hr, after which the larvae were removed. The plants were then sprayed with either the treatment or the control. The groups of plants were replaced twice during the experiment.

Undamaged Plants and Filter Paper. To investigate whether botanical pesticides influence parasitoid behavior, irrespective of the presence of damage, undamaged plants were also compared. Three doses of the syringa extract were tested: 1, 3, and 5 g (see description above for preparation of the extracts), and one dose of neem. Finally, a test was performed to investigate whether the syringa extract alone influenced parasitoid behavior. For this test, filter paper was dipped into either the 5-g syringa treatment or into the control, and parasitoid behavior was observed.

Analysis of Plant Volatiles. To investigate differences in volatile emission by cabbage plants (*Brassica oleracea* var. *gemmifera* cv. Cyrus) that had been treated with syringa extracts and control plants, headspace analysis was carried out. The 5-g syringa extract was made as described above, and 100 ml were applied to each clean, undamaged, cabbage plant. Plants that served as controls were sprayed with 100 ml of distilled water mixed with liquid detergent. The plants were left to dry for 60 min before being placed into 30-l collecting jars.

Pressurized air was filtered over silica gel, molecular sieves (8–12 mesh beads, 4-Å pore width, Sigma), activated charcoal, and a disposable Tenax-containing tube (90 mg Tenax TA) before entering the collecting jar. The air-inlet, air-outlet, filters, and sampling jars were connected with 0.8-cm diam Teflon tubing. Prior to the experiments and between each sample, the system was purged with purified air overnight at a flow rate of 500 ml/min.

Cabbage plants were carefully removed from the pots, taking care not to disturb the root system; then the entire soil and root system was covered in

aluminum foil. Plants were placed individually into the collecting jars, which were covered with a loose glass lid. A viton O-ring was placed between the jar and the lid, and the lid was tightly closed with a metal clamp. High-frequency fluorescent lights (30–35 μmol photons/m^2/sec) were placed 15 cm above the collection jars. The system with the plants was purged for 1 hr at 500 ml/min, after which the flow was reduced to 225 ml/min, and a Tenax (90 mg Tenax TA) trap was connected to the air outlet in the lid of the collecting jar. Volatiles were trapped at a rate of 175 ml/min by pulling the air through the trap by using an in-house vacuum. Volatiles were collected for 4.5 hr, after which the traps were refrigerated (±4°C) until they were analyzed. Four independent samples were collected for each treatment (5-g syringa and control). These independent samples were collected on consecutive days, and each was collected in the morning at the same time each day. After the second sample, and again after the final sample, two blank samples were taken from empty collecting jars to ensure that volatiles always present in filtered/clean air were not considered in the final analysis.

Samples of volatiles were also taken from the syringa extract mixed with liquid detergent. For this sample, 25 ml of extract were placed into a glass Petri dish and placed on top of an Erlenmeyer flask to provide sampling at approximately the same height as the cabbage plant. The Petri dishes were placed into the collecting jars, and the system was purged for 1 hr. After 1 hr, the airflow was reduced to 225 ml/min. A Tenax TA trap was used to collect the volatiles, and air was pulled through the trap at 175 ml/min. Volatiles were collected for 1 hr, after which the Tenax traps were refrigerated (±4°C) until they were analyzed. Four samples were collected from the syringa extract.

Volatiles were released from the Tenax traps with a thermodesorption cold trap setup (Markes, UK) by heating at 200°C for 10 min, with a He-flow of 30 ml/min. Desorbed volatiles were collected in the cold trap at −100°C. Volatiles were injected in splitless mode into the RTX-5Silms column (Restec, 30 m × 0.32 mm ID, 0.33-μm film thickness) by heating the cold trap to 270°C. After an initial column temperature of 40°C for 2 min, the temperature was raised to 95°C at 3°C/min, then to 165°C at 2°C/min, and subsequently to 250°C at 15°C/min. The column was directly coupled to the ion source of a Finnigan quadrupole mass spectrometer, which was operating in the 70-eV EI ionization mode and scanning from mass 33 to 300 at 3 scans/sec. Compounds were identified by comparison of mass spectra with those in the NIST 98 and Wiley 7th edition spectral libraries and by checking the retention indices with those of authentic reference compounds.

For each compound, the mean peak area was calculated, and each compound was classified as being emitted in larger amounts by control or syringa-treated cabbage plants, based on mean peak area. A sign test was used to determine whether the number of compounds that were emitted in larger amounts by control or syringa-treated plants differed from a 50–50 distribution over the two treatments

(Sokal and Rohlf, 1995). To compare the total headspace composition of plants with and without syringa extract treatment, a principal component analysis (PCA) was carried out according to the description by Mumm et al. (2004).

RESULTS

Plant–Host Complex. Cabbage plants were sprayed with the botanical extracts or control solution and then infested with *Plutella xylostella*. Parasitoids were exposed to the entire plant–host complex in the wind tunnel. *Cotesia plutellae* did not show a preference for the treated or control plants when the plants were treated with the neem extract ($P = 0.093$; Figure 2). However, they did show a preference for plants that had been treated with the 5-g syringa extract ($P < 0.001$; Figure 2). When lower doses of the syringa extract were tested, *C. plutellae* still showed a clear preference for the 3-g syringa dose ($P = 0.029$). Differences were not significant at the lowest 1-g dose ($P = 0.052$; Figure 2). For further experiments with *C. plutellae*, the highest syringa dose (5 g) was used.

Diadromus collaris did not show a preference for the control or the treated cabbage plant for either of the botanical pesticides [neem, $P = 0.37$; syringa (5-g dose), $P = 0.36$].

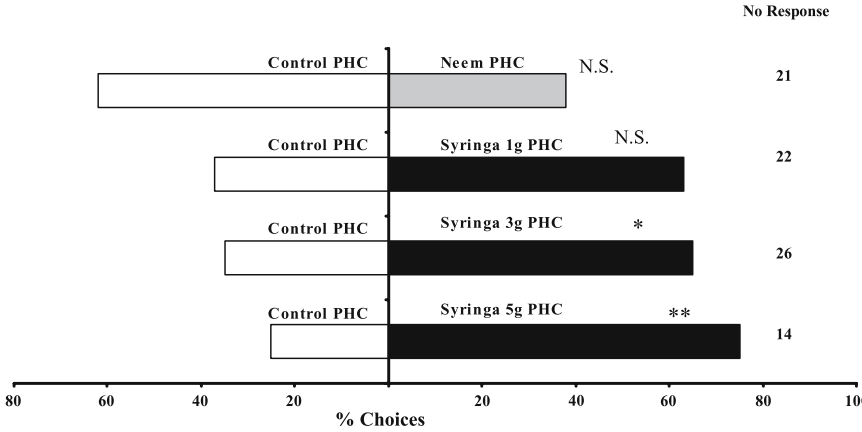

FIG. 2. Effect of syringa extract and neem on the response of female *Cotesia plutellae* to cabbage plants infested with *Plutella xylostella*. Each plant was sprayed with the control or the botanical pesticide and then infested with *P. xylostella*. Parasitoids were exposed to the entire plant–host complex (PHC). Percentage indicates total number of landings on target per treatment group (no. of responding wasps = 60). Significant differences are indicated in the graph (binomial test, N.S., $P > 0.05$; $*P < 0.05$; $***P < 0.001$). Number of females not responding is indicated on the right side of the figure.

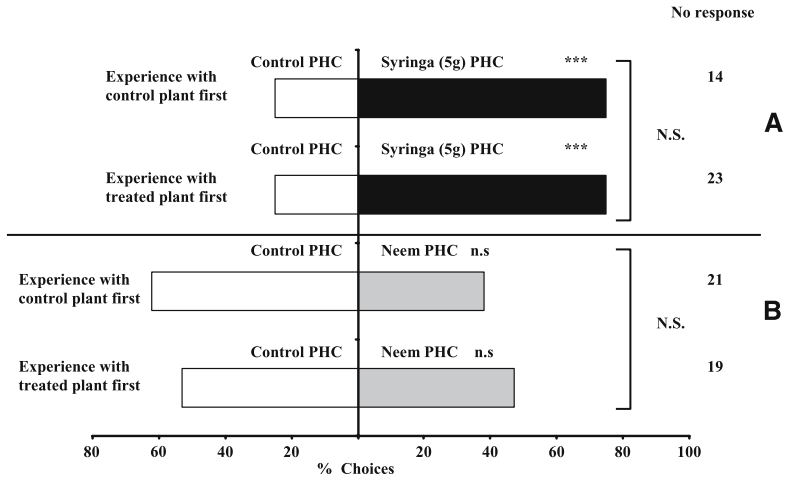

FIG. 3. Effect of previous experience on the response of female *Cotesia plutellae* to cabbage plants infested with *Plutella xylostella*. (A) Response to plants treated with syringa extract (5-g dose); (B) response to plants treated with neem. Each plant was sprayed with the control or the botanical pesticide and then infested with *P. xylostella*. Parasitoids were exposed to the entire PHC. Percentage indicates total number of landings on target per treatment group (no. of responding wasps = 60). Significant differences are indicated in the graph (χ^2, N.S., $P > 0.05$; binomial test: ns $P > 0.05$; ***$P < 0.001$). Number of females not responding is indicated on the right side of the figure.

Effect of Experience. The previous experience of female *Cotesia plutellae* with treated or control cabbage plants did not affect the response (neem, $\chi^2 = 0.93$, $df = 1$, $P = 0.64$; syringa, $\chi^2 = 1.679$, $df = 1$, $P = 0.44$; Figure 3A and B). Similar results were found for *Diadromus collaris*; the previous experience of the female with treated or control cabbage plants did not influence the subsequent behavior (neem, $\chi^2 = 0.031$, $df = 1$, $P = 0.97$; syringa, $\chi^2 = 0.304$, $df = 1$, $P = 0.86$; Figure 4A and B).

Response of Pupal Parasitoid. Diadromus collaris did not respond well in the wind tunnel, which is clear from the large number of "no responses" (±50%; Figure 4A and B). When damaged and undamaged cabbage plants were compared, females did not distinguish between them ($P = 1.0$), and less than 50% responded (Figure 4C). We, therefore, abandoned this parasitoid species for the remaining trials.

Damaged Plants without Hosts. Plants were sprayed with the botanical extract or control solution and damaged by *Plutella xylostella*, but before exposure to *Cotesia plutellae* in the wind tunnel, the *P. xylostella* larvae were removed. The removal of the hosts did not alter the response of the parasitoid.

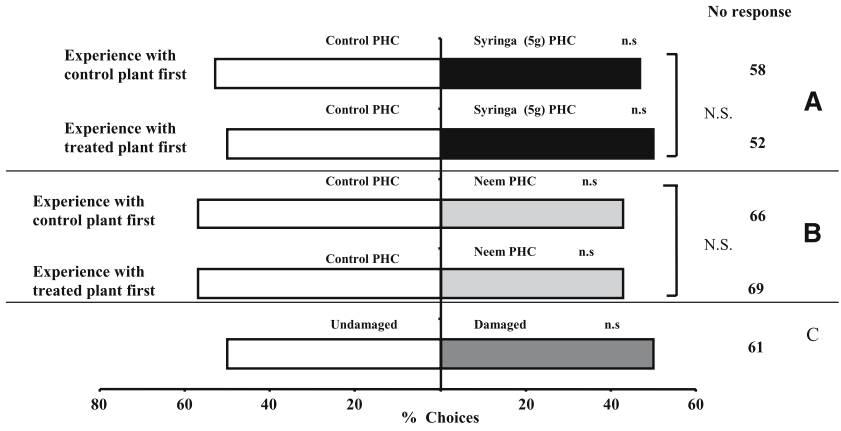

FIG. 4. Effect of previous experience on the response of female *Diadromus collaris* to cabbage plants infested with *Plutella xylostella*. (A) Response to plants treated with syringa extract (5-g dose); (B) response to plants treated with neem. Each plant was sprayed with the control or the botanical pesticide and then infested with *P. xylostella*. Parasitoids were exposed to the entire PHC. (C) Caterpillar damaged vs. undamaged cabbage plants; these plants were not sprayed with any treatment. Percentage indicates total number of landings on target per treatment group (no. of responding wasps = 60). Significant differences are indicated in the graph (χ^2, N.S., $P > 0.05$; binomial test: ns, $P > 0.05$). Number of females not responding is indicated on the right side of the figure.

Again, *C. plutellae* showed a preference for the plants that had been treated with the syringa extract ($P < 0.001$), whereas they did not for the treated or control plants if the plants had been sprayed with the neem extract ($P = 0.52$; Figure 5A).

Equally Damaged Plants without Hosts. To create equally damaged plants, the plants were infested with *Plutella xylostella* larvae for 24 hr, larvae were removed, and then plants were sprayed with botanical extract or control solution. *Cotesia plutellae* did not show a preference for the treated or the control plant when the plants had been treated with the neem extract ($P = 0.7$), but they again showed a highly significant preference for plants that had been treated with syringa extract ($P < 0.001$; Figure 5B).

Undamaged Plants and Filter Paper. Cotesia plutellae was exposed to undamaged cabbage plants that had been sprayed with the botanical extracts or the control, or to filter paper that had been dipped in the treatments. Despite the lack of damage, *C. plutellae* still showed a preference for plants that had been treated with the syringa extract, except at the lowest dose (1-g dose, $P = 0.092$; 3-g dose, $P = 0.027$; 5-g dose, $P < 0.001$). Again, there was no effect of treatment with the neem solution ($P = 0.52$; Figure 6). The number of females

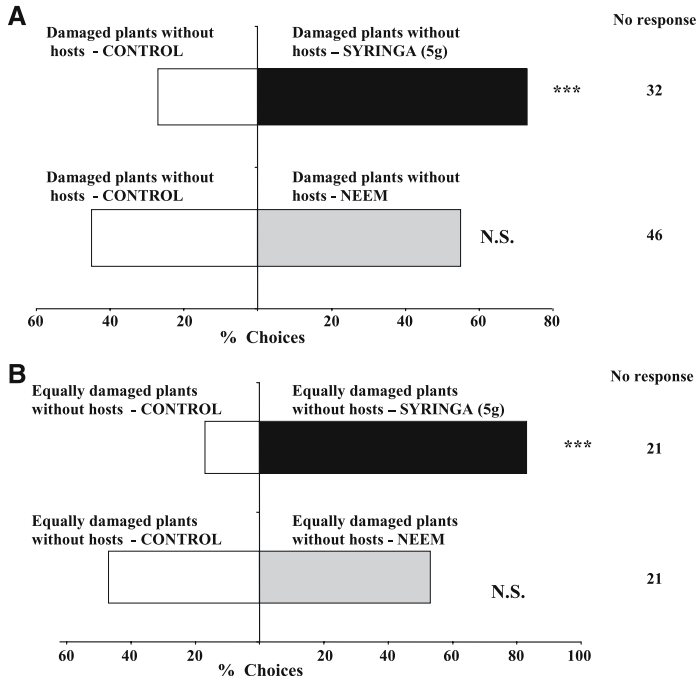

FIG. 5. Effect of syringa (5-g dose) and neem on the response of female *Cotesia plutellae* to treated cabbage plants. Percentage indicates total number of landings on target per treatment group (no. of responding wasps = 60). Significant differences are indicated in the graph (binomial test, N.S., $P > 0.05$; ***$P < 0.001$). Number of females not responding is indicated on the right side of the figure. (A) Cabbage plants had previously been infested with *Plutella xylostella* hosts. Each plant was sprayed with the control or the botanical pesticide and then infested with *P. xylostella*. The *P. xylostella* hosts were removed just before the cabbage plants were exposed to the parasitoids. (B) Cabbage plants were equally damaged. Each plant was infested with *P. xylostella* larvae for 24 hr, the larvae were removed, and then the plants were sprayed with the botanical pesticide or the control.

responding to undamaged plants was less than the number responding to caterpillar-damaged plants.

The attraction of the parasitoid to the syringa extract was lost when it was offered on filter paper instead of cabbage plants ($P = 0.70$; Figure 6).

Analysis of Plant Volatiles. Only compounds that were detected in two or more samples per treatment were included in the analysis. Headspace compositions of the control cabbage and syringa-treated cabbage were clearly different. The PCA resulted in a model with three significant principal factors,

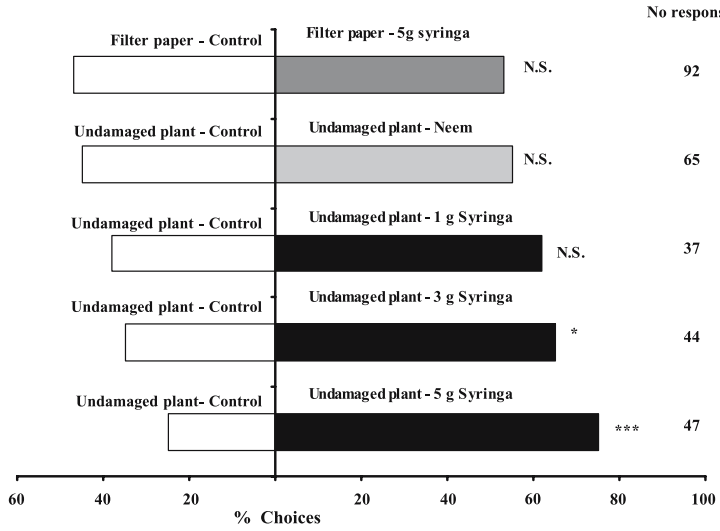

FIG. 6. Effect of syringa and neem on the response of female *Cotesia plutellae* to undamaged cabbage plants or to filter paper dipped in the highest dose of the syringa treatment. Each plant was sprayed with the control or the botanical pesticide, but plants were not infested with *Plutella xylostella*. The filter paper was dipped into the control or the syringa extract (5-g dose). Percentage indicates total number of landings on target per treatment group (no. of responding wasps = 60). Significant differences are indicated in the graph (binomial test, N.S. $P > 0.05$; *$P < 0.05$; ***$P < 0.001$). Number of females not responding is indicated on the right side of the figure.

explaining 72.5% of the variance in the data (Figure 7). A total of 49 compounds, alcohols, aldehydes, ketones, esters, terpenoids, sulfides, and an isothiocyanate were found in both control and treated plants (Table 1). Fifteen of the volatiles in the headspace from cabbage plants that had been treated with the syringa extract were also present in the syringa extract itself: four alcohols, two aldehydes, two esters, two ketones, three terpenoids, and two unidentified compounds (Table 1). Six additional compounds were found in plants that had been treated with syringa extract but not in control cabbage plants (Table 1). The syringa extract itself produced 25 compounds that were not found in the control plants or in the cabbage plants that had been treated with the syringa extract (Table 2). The total quantities of volatiles produced by plants that had been treated with the syringa extract were significantly higher than the quantities produced by control plants: of the 49 compounds, 38 compounds were emitted in larger quantities (based on mean peak areas) by syringa treated plants, and 11 were emitted in larger quantities by control plants (sign test, $P < 0.001$).

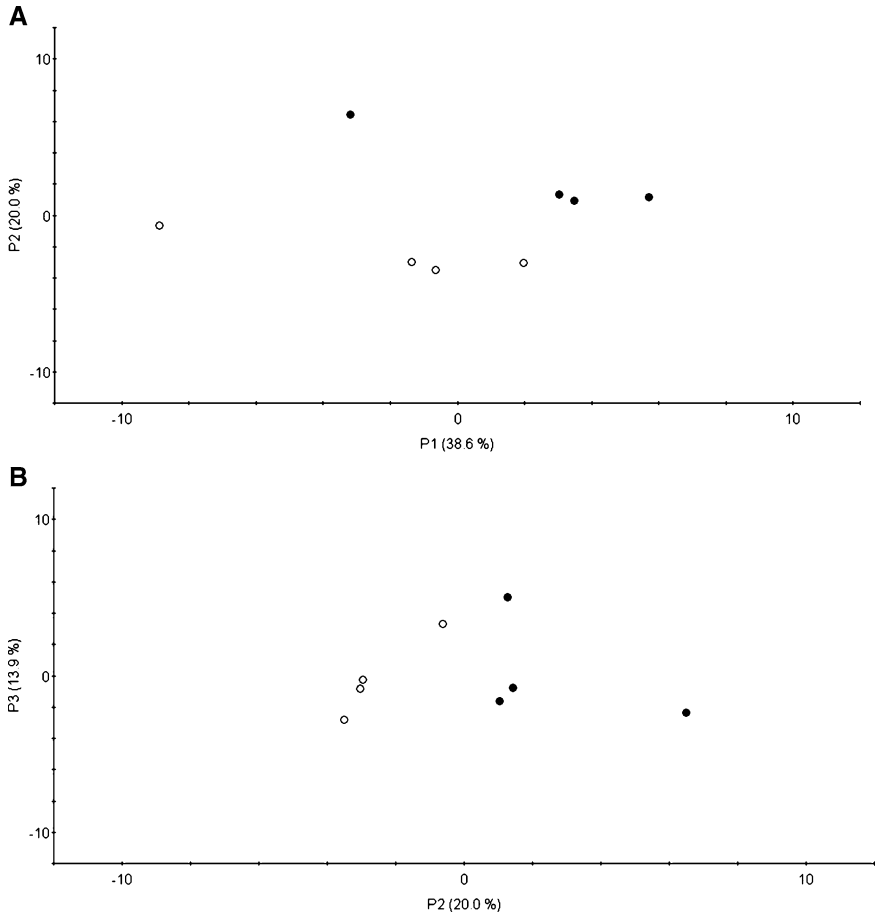

FIG. 7. Principal component analysis of the volatile patterns of differently treated cabbage plants. Score plots for the first (P1) vs. second (P2) significant principle factor (A) and for the second (P2) vs. the third (P3) significant principle factor (B) from partial-least-squares discriminant analysis based on absolute amounts of volatile constituents. Open circles represent samples of control cabbage plants, and filled circles represent samples for syringa-treated cabbage plants.

DISCUSSION

After an oviposition experience, *Cotesia plutellae* responded well in the wind tunnel. Females did not distinguish between cabbage plants that had been treated with the neem solution and plants that had been sprayed with the control.

TABLE 1. MEAN (±SE) OF GC PEAK AREA (ARBITRARY UNITS) FOR COMPOUNDS DETECTED IN HEADSPACE OF CONTROL CABBAGE PLANTS AND CABBAGE PLANTS TREATED WITH AQUEOUS LEAF EXTRACTS FROM *Melia Azedarach*

Number	Cabbage volatiles	Control cabbage	*N*	Treated cabbage	*N*	Syringa extract	*N*
	Alcohols						
1	1-Hexanol[a]	8.30 ± 3.33	4	13.00 ± 1.15	3	69.00 ± 11.63	4
2	2-Ethyl hexanol[a]	624.00 ± 89.54	4	807.50 ± 3.50	2		
3	1-Pentanol[a]	4.25 ± 1.11	4	7.53 ± 0.32	4	100.75 ± 22.35	4
4	1-Penten-3-ol[a]	193.75 ± 69.28	4	339.25 ± 7.23	4	459.50 ± 47.06	4
5	3-Hexen-1-ol[a]	69.25 ± 29.68	4	113.50 ± 12.58	4	3.08 ± 0.46	4
	Aldehydes						
6	2-Ethyl hexanal[a]	52.75 ± 8.67	4	177.50 ± 66.19	4	364 ± 43.58	4
7	Hexanal[a]	9.25 ± 1.38	4	14.50 ± 1.55	4	343.50 ± 69.50	4
	Esters						
8	1-Butanol-3methyl acetate	2.65 ± 1.05	2	2.38 ± 0.60	4		
9	2-Penten-1-ol acetate[a]	47.10 ± 24.15	4	92.00 ± 16.05	4		
10	3-Hexen-1-ol acetate[a]	420.25 ± 116.57	4	608.75 ± 39.70	4	10.57 ± 2.69	3
11	3-Hexen-1-ol-propanoate[a]	1.13 ± 0.38	3	2.45 ± 0.75	2		
12	2-Ethyl acetate[a]	580.25 ± 86.18	4	733.20 ± 410.67	3		
13	Acetic acid pentyl ester[a]	2.95 ± 0.91	4	6.15 ± 0.85	2	11.4 ± 3.29	4
14	Heptyl acetate[a]	2.25 ± 0.67	4	7.03 ± 0.54	3		
15	Methyl salicylate[a]	2.40 ± 1.00	3	5.25 ± 2.21	4		
	Isothiocyanate						
16	Methyl isothiocyanate	8.65 ± 2.25	4	8.33 ± 1.09	4		
	Ketones						
17	2-Heptanone[a]	3.75 ± 0.75	2	10.03 ± 1.23	4	36.25 ± 8.06	4
18	3-Heptanone[a]	15.75 ± 3.17	4	52.50 ± 10.84	4	311.75 ± 49.43	4
19	3-Pentanone[a]	101.75 ± 38.10	4	244.50 ± 32.85	4		
	Sulfides						
20	Dimethyl disulfide[a]	28.50 ± 12.51	4	42.75 ± 6.61	4		
21	Dimethyl trisulfide[a]	4.05 ± 1.41	4	10.68 ± 2.90	4		
	Terpenoids						
22	1,8-Cineole	167.00 ± 52.25	4	150.00 ± 30.82	4	1.03 ± 0.23	4
23	2-β-Pinene	77.75 ± 22.40	4	57.75 ± 8.81	4		
24	α-Phellandrene[a]	1.73 ± 0.32	3	18.35 ± 17.22	4		

TABLE 1. CONTINUED

Number	Cabbage volatiles	Control cabbage	*N*	Treated cabbage	*N*	Syringa extract	*N*
25	α-Terpinolene[a]	0.70 ± 0.15	3	15.63 ± 15.18	3		
26	α-Pinene	201.00 ± 57.79	4	186.50 ± 37.38	4		
27	α-Thujene	206.25 ± 63.60	4	185.75 ± 30.03	4		
28	β-Myrcene[a]	5.73 ± 1.07	3	33.28 ± 29.58	4		
29	γ-Terpinene[a]	295.50 ± 53.45	4	303.75 ± 41.35	4	5.25 ± 0.55	2
30	l-Limonene	4.15 ± 0.55	2	3.23 ± 0.35	3		
31	Mix α-terpineol	507.00 ± 76.95	4	392.50 ± 111.45	4		
32	(*E*)-β-Ocimene[a]	4.00 ± 1.56	4	7.98 ± 4.39	4		
33	α-Terpinene[a]	2.15 ± 0.15	2	2.58 ± 0.32	4		
34	Sabinene[a]	2.60 ± 0.45	4	7.48 ± 3.58	4		
35	*trans*-4-thujanol	17.25 ± 5.57	4	13.57 ± 2.43	3		
36	Unknown terpenoid 1	106.00 ± 28.54	4	81.00 ± 11.60	4	4.93 ± 0.77	4
	Others						
37	Unknown 1[a]	28.00 ± 1.00	3	44.67 ± 13.68	3		
38	Unknown 2[a]	1.38 ± 0.25	4	2.25 ± 0.38	4		
39	Unknown 3[a]	56.75 ± 23.78	4	105.75 ± 24.87	4		
40	Unknown 4[a]	8.30 ± 0.85	3	27.25 ± 3.30	4		
41	Unknown 5[a]	23.00 ± 8.74	3	40.67 ± 12.99	3	12.50 ± 0.50	2
42	Unknown 6[a]	4.93 ± 1.07	3	6.70 ± 2.10	2		
43	Unknown 7	1.20 ± 0.20	2	1.03 ± 0.17	4		
44	Unknown 8[a]	1.47 ± 0.26	3	5.77 ± 0.23	3		
45	Unknown 9[a]	25.67 ± 15.27	2	32.70 ± 18.18	3		
46	Unknown 10[a]	27.33 ± 11.26	3	29.63 ± 15.65	3		
47	Unknown 11[a]	51.67 ± 9.60	3	481.33 ± 150.93	3	631.67 ± 65.17	3
48	Unknown 12[a]	13.00 ± 4.56	4	28.00 ± 3.46	3		
49	Unknown 13[a]	17.25 ± 8.02	4	31.67 ± 4.48	3		
	Compounds only found in cabbage plants treated with syringa				*N*		
1	Methyl pyrrole			18.40 ± 11.60	2		
2	3-Methyl butanal			9.00 ± 1.57	3		
3	Quinhydrone			6.83 ± 2.04	4		
4	1-Octen-3-ol			4.23 ± 0.69	3		
5	Benzoxazole			2.65 ± 0.75	2		
6	*trans*-Carryophyllene			1.85 ± 0.55	2		

N = no. of samples out of 4, in which the compound had been identified.
[a]Compounds emitted in larger amounts by syringa-treated plants (sign test, $P < 0.001$).

TABLE 2. MEAN (±SE) OF GC PEAK AREAS (ARBITRARY UNITS) FOR COMPOUNDS DETECTED IN HEADSPACE OF SYRINGA EXTRACT ONLY

Number	Compounds only in Syringa	Syringa	*N*
	Alcohols		
1	3-Methyl-1-butanol	34.00 ± 8.33	3
2	1-Nonanol	11.13 ± 2.58	4
	Aldehydes		
3	(*E*)-2-Hexenal	8.58 ± 2.00	4
4	2-Pentenal	124.75 ± 31.68	4
	Ketone		
5	6-Methyl-5-hepten-2-one	184.50 ± 41.05	4
	Others		
6	Unknown 14	18.15 ± 4.66	4
7	Unknown 15	24.00 ± 5.03	3
8	Unknown 16	116.25 ± 27.16	4
9	Unknown 17	52.50 ± 10.25	4
10	Unknown 18	132.75 ± 10.35	4
11	Unknown 19	3.43 ± 1.15	3
12	Unknown 20	11.00 ± 1.00	3
13	Unknown 21	16.53 ± 8.24	3
14	Unknown 22	8.47 ± 3.50	3
15	Unknown 23	12.93 ± 2.15	3
16	Unknown 24	11.60 ± 3.24	3
17	Unknown 25	13.25 ± 2.21	4
18	Unknown 26	19.33 ± 0.67	3
19	Unknown 27	1.33 ± 0.35	4
20	Unknown 28	311.50 ± 57.12	4
21	Unknown 29	3.43 ± 1.04	4
22	Unknown 30	4.98 ± 1.27	4
23	Unknown 31	483.00 ± 84.00	2
24	Unknown terpenoid 2	23.50 ± 5.24	4
25	Unknown terpenoid 3	4.60 ± 3.70	3

N = no. of samples out of 4, in which the compound had been identified.

However, *C. plutellae* always preferred cabbage plants that had been sprayed with the syringa extract to the control plants, except at the lowest dose. *C. plutellae* appears to detect and respond differently to volatiles from plants treated with these two botanical extracts. Akol et al. (2003) have shown that *Diadegma mollipla* is able to detect and distinguish between volatiles emitted by cabbage plants sprayed with two different neem formulations. In their experiment, they found that a neem seed oil formulation had a negative effect on the foraging of *D. mollipla*, as females significantly preferred volatiles from control plants over those from plants sprayed with neem formulation. However, when *D. mollipla* had a choice between plants sprayed with a solution from a neem kernel cake powder and control plants, they did not distinguish between

sprayed and control plants (Akol et al., 2003). *Uscana lariophaga*, an egg parasitoid of the bruchid *Callosobruchus maculatus*, was repelled by neem seed oils on cowpea beans, but the larval parasitoid *Dinarmus basalis* did not discriminate between control and neem-treated beans (Boeke, 2002; Boeke et al., 2003). In our study, the neem formulation Neemix 4.5® did not appear to have an adverse effect on foraging by *C. plutellae*.

Pre-flight experience can have a distinct effect on parasitoid behavior (reviewed by Turlings et al., 1993 and Vet et al., 1995). However, learning is thought to have a less important role for specialist parasitoids (Geervliet et al., 1998). Both *Cotesia plutellae* and *Diadromus collaris* are relatively specialized parasitoids attacking mainly *Plutella xylostella*, and results from this study show no indication that these parasitoids acquired a response to syringa through associative learning.

The response of *Cotesia plutellae* to volatiles from herbivore-damaged plants that had been treated with syringa extract was independent of the presence of host larvae. Previous studies have also shown that herbivore-damaged plants are attractive to *C. plutellae* (Shiojiri et al., 2001; Vuorinen et al., 2004) even after removal of the hosts (Potting et al., 1999).

Schuler et al. (1999, 2003) found that the amount of damage was an important factor influencing the flight responses of *Cotesia plutellae*. In previous studies, we have shown that *Plutella xylostella* damage is lower on cabbage plants treated with botanical extracts (Charleston, 2004). However, our data show that the response of *C. plutellae* was similar whether the cabbage plants had been sprayed with the botanical extracts first or whether plants were first damaged by *P. xylostella* larvae and then sprayed with the botanical extract. The difference in feeding damage between the treated and control cabbages may have been too small to affect the flight response of *C. plutellae*.

The pupal parasitoid *Diadromus collaris* was not attracted to volatiles emitted by cabbage plants in the wind tunnel and did not distinguish between caterpillar-damaged and undamaged plants. *D. collaris* is a pupal parasitoid, and while there is a wealth of knowledge available on the role of volatile cues used by parasitoids attacking larval stages of herbivores (Dicke and Vet, 1999), there is not much information about the chemical cues used by pupal parasitoids (Vet et al., 1995). Some pupal parasitoids have been shown to respond to plant volatiles. The stemborer pupal parasitoid *Dentichasmias busseolae*, for example, makes use of plant volatiles from maize and sorghum and is particularly attracted to herbivore-damaged plants (Gohole et al., 2003). For pupal parasitoids, as for all parasitoids, the possibilities of using direct host-derived cues are limited, and in addition, larvae often pupate away from the site of damage, restricting the use of predictable indirect cues by pupal parasitoids (Vet et al., 1995). The opportunity of using volatiles from larval feeding damage is limited to situations where the pupae stay in or on the plant, and larval and pupal stages co-occur. *Plutella xylostella* tend to pupate on the plant (although not directly

near feeding damage), and, because of fast development and overlapping generations, larval and pupal stages are often found together in the field. Hence, *D. collaris* could make use of plant volatiles to find its host. Electroantennogram (EAG) studies indicate that *D. collaris* does respond to cabbage volatiles (Lecomte and Pouzat, 1985), which further suggests that this parasitoid may make use of plant volatiles. However, in our studies, *D. collaris* did not appear to use herbivore-induced plant volatiles. Therefore, it is possible that *D. collaris* makes use of other strategies to find its host. *Cotesia plutellae* was attracted to cabbage plants treated with syringa extract. Even undamaged cabbage plants treated with syringa extract attracted the parasitoids. In contrast, *C. plutellae* did not show a preference for filter paper dipped in the syringa extract. Headspace analysis yielded a total of 49 compounds from both undamaged control cabbage plants and undamaged cabbage plants that had been treated with syringa extract. The volatile bouquet emitted after treatment with syringa extract is composed of the same components as emitted by untreated cabbage plants. This is comparable to what happens in response to herbivory: the composition of the bouquet is qualitatively similar to that emitted by intact or mechanically damaged plants, and consists of fatty acid derivatives, terpenoids, and a few sulfur-containing compounds such as methyl isothiocyanate and sulfides. However, the quantity of volatiles was significantly higher in the plants treated with the syringa extract. Some of the volatiles found in cabbage plants treated with the syringa extract were also present in the syringa extract itself. Yet, many of the volatiles emitted by the syringa extract were not emitted, or were only emitted at low rates, from cabbage plants treated with the syringa extract. This indicates that the enhanced emission of the plant volatiles from cabbage plants treated with the extract is caused by an induction of these volatiles in cabbage rather than an evaporation of the syringa extract from the treated plants. To our knowledge this is the first example of a plant extract causing an increased emission of plant volatiles, which is likely to explain the significant attraction of *C. plutellae* to plants that have been treated with the syringa extract. Which of the emitted volatiles attract the parasitoids remains to be elucidated. For crucifer–parasitoid interactions, a gas chromatography (GC)–EAG approach has limited the total number of compounds to be tested in behavioral tests to about 20 for the parasitoids *Cotesia glomerata* and *Cotesia rubecula* (Smid et al., 2002). A recent field study in an open field and a hop yard showed that 11 carnivorous arthropods were attracted to 13 herbivore-induced plant volatiles (James, 2005).

It is well-known that elicitors can induce parasitoid-attracting plant volatiles. Among these elicitors are, for example, compounds from herbivore regurgitant (Alborn et al., 1997; Mattiacci et al., 1995) or phytohormones (Dicke et al., 1999; Gols et al., 1999; Ozawa et al., 2000; Horiuchi et al., 2001). Furthermore, there is evidence that plants can affect the emission of volatiles in downwind neighboring plants (Dicke and Bruin, 2001; Pichersky and

Gershenzon, 2002; Choh et al., 2004; Engelberth et al., 2004). Some green leaf volatiles, i.e., (*Z*)-3-hexenol, (*E*)-2-hexenal, and (*Z*)-3-hexenyl acetate, induce gene expression in plants that are exposed to these compounds in an airtight container (Arimura et al., 2001). We have recorded (*E*)-2-hexenal in the headspace of the syringa extract. However, whether this compound is responsible for inducing the emission of parasitoid attractants in cabbage remains to be investigated.

Our data show that treatment of cabbage with syringa extract or Neemix 4.5® would not impair the process of host habitat location by *Cotesia plutellae* or *Diadromus collaris*. In fact, *C. plutellae* was always attracted to cabbage plants treated with the syringa extract, even when the plants were undamaged, which may indicate an interaction between a plant and a botanical extract that enhances natural enemy activity. This may have negative implications for biological control. If the parasitoids do not discriminate between infested and uninfested plants, then they may waste time searching uninfested plants, which may result in a reduction of the parasitization rate (Dicke et al., 1990). Treatment of plants with the plant hormone jasmonic acid results in an increased emission of plant volatiles, and this results in an attraction of parasitoids and predators (Dicke et al., 1999; Gols et al., 1999, 2003; Thaler, 1999; Ozawa et al., 2004). Yet, in field-grown tomatoes, a blanket treatment with jasmonic acid resulted in higher parasitization levels of *Spodoptera exigua* compared to control plots (Thaler, 1999). In our study, the parasitoids were only given a short preoviposition experience with cabbage leaves that were infested with host insects and were not given the opportunity to discriminate between damaged and undamaged plants. This is not representative for most foraging decisions in nature, where previous foraging experiences, both positive and negative, can be integrated (Vet et al., 1998; Dicke, 1999b). Under field conditions, infested and uninfested plants are likely to be in close proximity, and the parasitoid may soon learn to discriminate. It would be interesting to expand these observations to investigate the impact that these botanical pesticides have on parasitoid foraging behavior in field situations.

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