

DEET Repels *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) Adults in Laboratory Bioassays¹

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Abstract The potential of least-toxic compounds to repel adults of the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), was explored. Bioassays in olfactometers and Petri dishes were designed to test the hypothesis that DEET (*N,N*-diethyl-3-methylbenzamide) can effectively repel *H. axyridis* adults. A bioassay in a Y-tube olfactometer indicated that beetles spent significantly less time in the test arm (DEET; 10, 100, and 1,000 µg) than in the control arm (hexane blank) within a 10-min time frame. A bioassay in a 3-neck bulb-tube olfactometer indicated that significantly more beetles avoided the test arm (DEET, 142 µg) than the control arm (hexane blank) within a 40-min time frame. A bioassay in a Petri dish revealed that significantly more beetles avoided filter paper disks treated with DEET (0.1 and 1.0 mg/cm²; aged for 1 d or 5 d) than acetone (the control) within a 60-min time frame. Finally, another bioassay in a Petri dish revealed that significantly more beetles avoided filter paper strips coated with DEET/paraffin (1% or 9% mixture, aged for 1 d or 23 d) than camphor/paraffin (0.1, 1 and 9% mixtures, aged for 1 d or 23 d), or paraffin alone (the control). This investigation suggests that DEET has good potential for repelling *H. axyridis* adults and should be field-tested on urban structures.

Key Words camphor, *N,N*-diethyl-*m*-toluamide, *Harmonia axyridis*, predator, repellents

Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae) is an entomophagous lady beetle originating in Asia. It has been introduced into eastern and southern United States, California, the Hawaiian Islands and Nova Scotia (Timberlake 1943, Gordon 1985, McClure 1987, Tedders and Schaefer 1994), Greece (Katsoyannos et al. 1997) and France (Ongagna et al. 1993). It was first recovered in North America in Louisiana and Mississippi (Chapin and Brou 1991), then in the southeastern states (Gordon and Vandenberg 1991, Tedders and Schaefer 1994) and western states (Dreistadt et al. 1995, LaMana and Miller 1996). Now, *H. axyridis* has expanded its range northward into most northern states and Canada (Day et al. 1994, Hoebeke and Wheeler 1996, McCorquodale 1998).

In spring and summer, *H. axyridis* larvae and adults are predators of aphids, scales and other soft-bodied insects on plants in forest and agricultural landscapes (McClure

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1986, Ferran et al. 1996, LaMana and Miller 1996, Trouve et al. 1997, Brown and Miller 1998, Michaud 2000, Brown 2003). In early to late fall, depending on geographic locality, adults migrate from feeding sites to overwintering sites. In Japan, adults pass the winter in mass aggregations within cracks and crevices of rock outcroppings as well as man-made structures (Obata 1986, Sakurai et al. 1993). In North America, adults also form winter aggregations in sheltered places, including buildings (Kidd et al. 1995, Nalepa et al. 1996, Nalepa et al. 2000, Schaefer 2003).

The propensity of adults to enter houses in the fall season has become a problem for homeowners. Beetles that successfully enter houses can aggregate by the thousands in secluded dark places (e.g., attics). During unseasonably warm days in winter, beetles often become active and pose a nuisance in interior living spaces, either by their mere presence or by reflex bleeding when crushed or handled roughly. Reflexed blood has an unpleasant odor and can stain walls, furniture and draperies. In addition, human allergic reactions (i.e., allergic rhinoconjunctivitis) to *H. axyridis* particulate inhalants (perhaps from dead beetles) have been reported (Yarbrough et al. 1999).

There is a need to discover effective non-toxic methods to thwart *H. axyridis* entry into buildings. Management of this nuisance pest could be achieved by a "push-pull" strategy (Riddick et al. 2000). This involves pushing beetles away from buildings with repellents, then pulling them into collecting vessels (e.g., traps) with attractants or persistent pheromones. Captured beetles could be cold-stored in depositories until the spring, then released into agricultural, forest or urban landscapes to serve as predators of plant pests. The push-pull strategy may also be useful for modifying the behavior of another house-invading lady beetle, *Adalia bipunctata* (L.). Adults of this species are known to overwinter in houses in urban areas in the United Kingdom (Benham and Muggleton 1978, Majerus 1994, 1997).

To date, research has centered on the identification of repellent chemicals. Riddick et al. (2000) examined the potential of plant-derived natural products to repel *H. axyridis* adults attempting to enter cracks or crevices in building exteriors. Camphor and menthol were the most effective of a range of monoterpenoids tested in olfactometer and Petri dish bioassays in the laboratory and camphor repelled beetles from treated surfaces in the field. For example, when camphor (9.4% emulsified concentrate) was sprayed into crevices on the exterior of a building through which beetles were entering, 100% of approaching beetles were repelled for the duration of the tests (0.5 h, 2 replicates). However, the repellent effect was short-lived because beetles were seen entering cracks 48 h post-treatment (Riddick et al. 2000). The repellent activity of camphor must be prolonged to be useful under field conditions and other putative repellents against *H. axyridis* need to be identified. Other recent research has examined the potential of conventional insecticides to kill *H. axyridis* adults that congregate on house exteriors (Williams et al. 2002b), and to repel or kill adults that are found feeding on injured grapes in vineyards (Williams and Fickle 2002, Williams et al. 2002a).

In this study, the effectiveness of DEET (*N,N*-diethyl-3-methylbenzamide, formerly known as *N,N*-diethyl-*m*-toluamide) as a repellent against adults of *H. axyridis* was evaluated. DEET effectively repels mosquitoes and other biting flies (Schreck 1977, Klun et al. 2003) and ticks (Dautel et al. 1999, Pretorius et al. 2003) from humans. DEET has been shown to repel Africanized honey bees, *Apis mellifera scutellata* Lepeletier, from humans (Collins et al. 1996) and stored product beetles from food and clothing (Watson and Barson 1996, Watson et al. 1997). DEET has never been

used to repel nuisance, but otherwise beneficial, lady beetles from the exterior surfaces of buildings. This research provides information for developing a bio-rational approach to managing *H. axyridis* populations in fall and winter seasons.

Materials and Methods

Insects. *Harmonia axyridis* adults were found aggregating on the inner walls of a watchtower near Lancaster, PA, in December 1995 and 1996. Each year, several thousand beetles were brushed from the inner walls and into plastic containers and transported in ice chests to the laboratory. Beetles were maintained in clear plastic, 3.8-liter containers with screened lids at a density of approximately 200 beetles per container. Each container was supplied with a cotton-stoppered water vial and honey (pure) smeared on the screened lid. Beetles were maintained in growth chambers at 15°C and 12:12 (L:D) photoperiod; once a week they were moved to room temperature for approximately 4 h, fed honey *ad libitum* and misted with water. Prior to behavioral testing, beetles were placed at room temperature in the laboratory for 1 to 2 h and again misted with water and fed. The age of beetles prior to testing was unknown.

Bioassay in γ -tube. Glass γ -tube olfactometers (~ 5 cm arms, 1 cm-i.d.) were used to compare the behavior of individual beetles that moved into test vs control arms in response to volatiles within a 10-min time frame. A strip of treated filter paper (2.0 × 0.5 cm, L × W; Whatman® grade 1) was placed within another glass tube (~ 2.5 cm, 1 cm-i.d.) that was fitted at the apical end of the test and control arms. Compressed house air was purified through activated charcoal (Sigma Chem. Co., St. Louis, MO) and humidified through a Dudley bubbling tube before entering test or control arms of the γ -tube olfactometer. All components were connected with Tygon® silicon tubing. The flow rate exiting the olfactometer was adjusted to 100 ml/min. The apparatus was positioned horizontally on a counter-top in the laboratory (at 23 ± 2.5°C) with bright, overhead fluorescent lights. Experiments were conducted between 1000 to 1700 h.

This experiment consisted of a completely randomized design with 7 to 10 replicates per concentration of test solutions. Test solutions were 0.1, 1, 10 and 100 µg/µl of DEET, in hexane. Experiments (with concentration) were conducted on 29 April 1997 (0.1 µg/µl), 25 April 1997 (1 µg/µl), 24 April 1997 (10 µg/µl), and 23 April 1997 (100 µg/µl). Because the four concentrations were tested on different dates, the response of beetles to each concentration was determined separately. Ten µl of the test concentrations or control (hexane blank) were applied to filter paper strips. After the treated strips were placed inside the γ -tube, a beetle was introduced into the apparatus, and air flow was restored throughout the apparatus. Test and control arms of the olfactometer were alternated after every replicate. We used four γ -tube olfactometers (all of approximately the same dimensions) interchangeably. Each olfactometer was washed in soapy water then dried and oven-baked at ≥100°C immediately after each replicate. The amount of time (in seconds) that each beetle remained in the test vs control arm was recorded per 10-min replicate. Each beetle was tested only once. Only male beetles were used.

Bioassay in 3-neck bulb-tube. Glass 3-neck bulb-tube olfactometers (Borges and Aldrich 1994) were used to compare the behavior of a group of beetles that moved into test vs control arms in response to airborne volatiles within a 40-min time frame. Test and control arms were 250-mL splash-guard adapters connected at their

lower joints to a 100-mL round-bottom, angled 3-neck distilling flask (Fig. 1). [Splash-guard adapters and the 3-neck distilling flask were purchased from Aldrich (Milwaukee, WI)]. The central neck of the distilling flask was fitted with an adapter connected to a vacuum via a rubber hose. Ambient air was drawn by vacuum through a Dudley bubbling-tube humidifier, Tygon silicone tubing, a glass filter (containing charcoal and glass wool) at the outer joint of test and control arms, then into the central cavity of the distilling flask and finally exiting through the central neck. The air flow rate was set at 100 mL/min for both test and control arms. The apparatus was positioned horizontally on a counter top in the laboratory (at $23 \pm 2.5^\circ\text{C}$) with bright, overhead fluorescent lights. Experiments were conducted between 1000 and 1700 h.

This experiment consisted of a completely randomized design with four replicate trials conducted on the same day, 13 March 1997. Two microliters of test or control chemicals were applied to filter paper disks (2.1 cm diam; Whatman® grade 1), and these were placed in either of the two arms of the olfactometer. The test solution was DEET (Aldrich, Milwaukee, WI) at $71 \mu\text{g}/\mu\text{l}$, in hexane. This concentration had been used previously to test the repellency of R-(+)-camphor in the same experimental design (Riddick et al. 2000). Hexane was included as a control. Twelve beetles were introduced at once into the central cavity of the distilling flask, through the central neck; the adapter was reattached to the central neck, and the airflow was restored to the apparatus.

Timing and observing beetle behavior began as soon as the vacuum was restored.

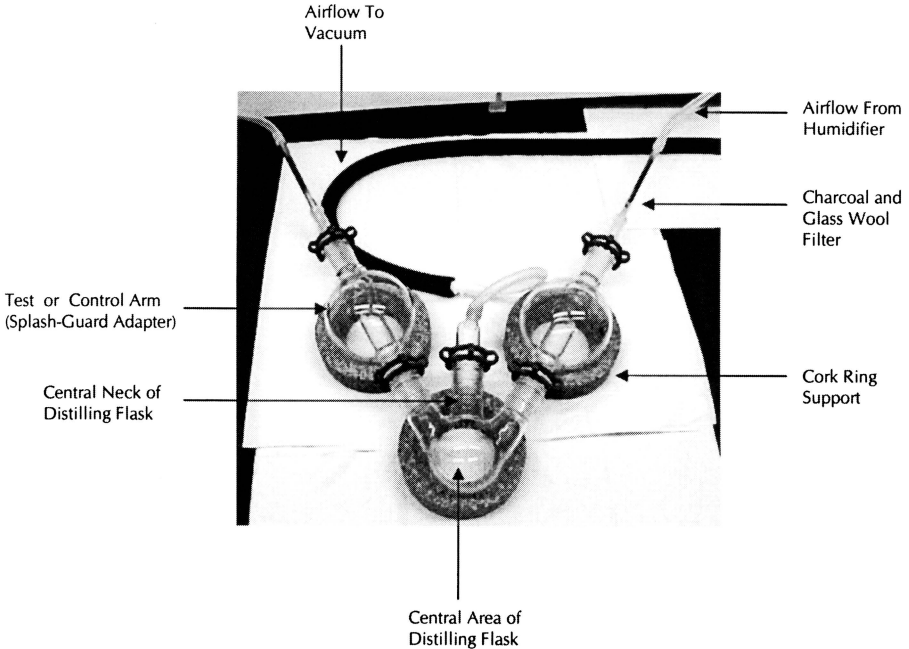


Fig. 1. Digital picture of a 3-neck bulb-tube olfactometer used to measure the behavioral responses of *H. axyridis* adults to volatiles of test vs control chemicals.

Beetles easily traversed the inner walls of the olfactometer, in both test and control side arms. Test and control arms were alternated after every trial. Two identical bulb-tube olfactometers were used interchangeably. Each olfactometer was washed in soapy water then dried and oven-baked at $\geq 100^{\circ}\text{C}$ between trials. The number of beetles in the test vs control arm of the olfactometer was determined at 5-min intervals for 40 min. Sex of beetles used in this experiment was not determined.

Bioassay in Petri dish with disk. Glass Petri dishes (20 mm deep, 150 mm diam) were used to measure the avoidance behavior of a group of beetles in response to three concentrations of DEET in relation to day after application (1 or 5 d). This experiment consisted of a completely randomized design with two replicate trials conducted on the same day, 22 March 1995. A filter paper disk (4.25 cm, diam; Whatman® grade 1) was treated with 200 μl of the test solution, at three concentrations (0.01, 0.1, or 1.0 mg/cm^2) plus the control (acetone blank) on the same day. Treated disks were set aside for 1 d or 5 d before being exposed to beetles. One Petri dish was used per treatment for a total of eight replicate dishes (or treated circles) per trial or 16 replicate dishes (or treated circles) for the entire experiment. Two trials were considered.

A gray-colored mesh screen (1.59 mm mesh size) served as the floor of the arena, and the inverted Petri dish bottom was the cover. Each treated disk was placed on top of the screen. One day after applying all concentrations of DEET and acetone to filter paper disks, approximately 20 μl of pure honey was applied to a filter paper strip (1.5 x 1.0 cm, L x W; Whatman® grade 1), affixed to the center of the treated circles. The honey served as a food attractant. Next, 10 unfed beetles (of undetermined hunger levels), were removed from cold storage, acclimated (for approximately 2 h) to ambient temperature (~ 21 to 23°C), and placed at random within the arenas. The number of beetles avoiding DEET-treated disks was determined at 10-min intervals. After 60 min, all beetles were removed from the test arenas and not used again. The same procedures were followed 5 d after chemicals had been applied to previously unused disks. The only exception was that a new filter paper disk was used for the control (acetone blank); it was used within 1 h after acetone was applied to it. The number of beetles avoiding the treated disks was determined at 10-min intervals for 60 min. Males were used in the first trial and females were used in the second trial.

Bioassay in Petri dish with strip. Glass Petri dishes (20 mm deep, 100 mm diam) were used to measure the avoidance behavior of individual beetles in response to DEET or R-(+)-camphor (Aldrich, Milwaukee, WI), both of which were formulated with melted paraffin (Paraplast™, Fisher Scientific; Pittsburgh, PA). This experiment compared the effects of three concentrations of DEET and camphor (0.1, 1, and 9%) and days after application (1 d vs 23 d) on the number of beetles repelled from a treated strip. A randomized block design was used with three replicate treated strips tested against a series of 10 beetles, for a total of 30 observations per treatment group per day.

Paraffin (24 to 25 ml) was heated in separate glass beakers until completely melted ($\geq 56^{\circ}\text{C}$). Then pre-measured amounts of camphor or DEET were added to the paraffin and gently stirred with a glass rod. The following mixtures were prepared: 9.4% D-P (2.5 ml DEET, 24 ml paraffin), 1% D-P (0.25 ml DEET, 25 ml paraffin), and 0.1% D-P (0.025 ml DEET, 25 ml paraffin), 9.1% C-P (2.5 g camphor, 25 ml paraffin), 1% C-P (0.25 g camphor, 25 ml paraffin), and 0.1% C-P (0.025 g camphor, 25 ml paraffin). While the mixtures were still warm, filter paper strips (9.0 x 1.0 cm, L x W; Whatman® grade 1) were randomly dipped into the liquid, so that all surfaces of the

paper were lightly wetted, then removed and placed in a clean glass Petri dish with lid, depending on chemical and concentration. All concentrations of DEET or camphor were applied to filter paper strips on the same day, 6 May 1997.

At the onset of experimentation, each treated strip was positioned and taped (underneath) to the edge of a 1/2 circle of filter paper then placed inside a clean glass Petri dish. Each Petri dish was placed horizontally under a chemical flow hood (with overhead, bright fluorescent lights, $23 \pm 2.5^\circ\text{C}$) before introducing a beetle. Each beetle was placed on the untreated 1/2 circle, and oriented in the direction of the treated strip. Three series of 10 beetles were tested per concentration of DEET or camphor. Each beetle was tested only once. Each treated strip was replaced with a new strip after each series of 10 beetles, and a separate, clean Petri dish was used for each series. Afterwards, each Petri dish was thoroughly washed in soapy water and then air-dried. The number of beetles (out of 10 per strip) displaying an avoidance response (jump back or turn away) was determined per chemical type and concentration on 7 May and 29 May 1997, 1 d and 23 d after application, respectively. Only male beetles were used in this experiment. Storage conditions before initiating the experiment and between 1 d and 23 d treatments were identical (15°C and 12:12 (L:D) photoperiod).

Statistical analysis. Paired *t*-tests were used to compare the avoidance response of beetles to volatiles of DEET in γ -tube and bulb-tube olfactometer bioassays. The general linear model (GLM) with analysis of variance (ANOVA) was used to detect a significant avoidance response to DEET concentration and days after application in the Petri dish with disk bioassay. GLM with ANOVA was also used to detect a significant avoidance response to DEET or camphor concentrations and days after application in the Petri dish with strip bioassay. Data were square-root transformed prior to analysis (Zar 1999). Means were considered significantly different at $P \leq 0.05$. When more than two treatments were compared, the Tukey HSD method was used for separation of means. Statistical analyses were performed with Systat (1998) or Sigma Stat (1994) computer software. Untransformed data are presented.

Results

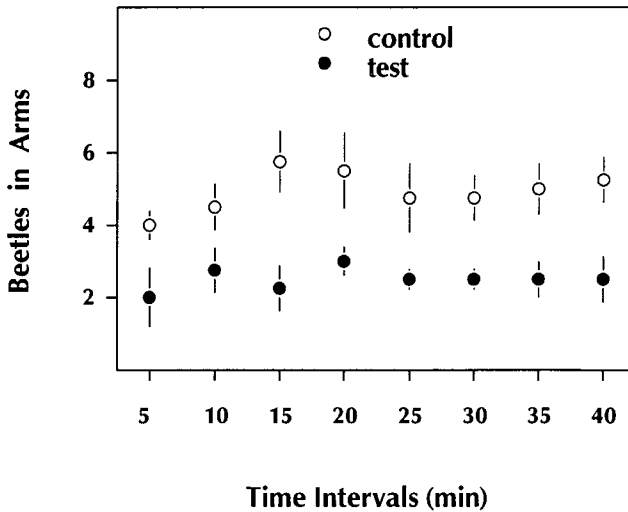
Bioassays in olfactometers. In the γ -tube olfactometer bioassay, significantly fewer beetles occupied the arm containing 10 μl of 1, 10 or 100 $\mu\text{g}/\mu\text{l}$ of DEET vs the control (Table 1). No difference was detected between 0.1 $\mu\text{g}/\mu\text{l}$ of DEET and the control. A comparison of behavioral responses between concentrations was not possible because all concentrations were not on the same day.

In the bulb-tube olfactometer bioassay, significantly fewer beetles occupied the side arm containing DEET than the side arm containing hexane (Fig. 2; paired *t*-test, $t = -8.0$, $df = 31$, $P < 0.0001$). The overall mean \pm SEM number of beetles in the side arm containing DEET and the side arm containing the control (hexane blank) was 2.5 ± 0.2 and 4.9 ± 0.25 per time interval, respectively. A maximum of 3.0 ± 0.8 beetles was present in the test arm at the 20-min interval; whereas, a maximum of 5.75 ± 1.7 beetles was present in the control arm at the 15-min interval.

Bioassay in Petri dish with disk. The Petri dish with disk bioassay indicated that the interaction between day after application and DEET concentration was significant ($F = 12.5$; $df = 3, 87$; $P < 0.0001$). Effects of day and concentration were significant when analyzed separately (day: $F = 11.6$; $df = 1, 87$; $P = 0.001$; concentration: $F = 63.4$; $df = 3, 87$; $P < 0.0001$). At 1 d after application, more beetles avoided disks

Table 1. Mean \pm SEM time (in seconds) spent by *H. axyridis* males in test vs control arms of a Y-tube olfactometer for four concentrations of DEET in 10-min trials

Concn ($\mu\text{g}/\mu\text{l}$)	Test arm	Control arm	Statistic*			
			<i>t</i>	df	<i>P</i>	n
0.1	143.7 \pm 24.0	81.9 \pm 16.9	1.84	6	0.11	7
1	43.2 \pm 14.0	169.7 \pm 34.7	-2.21	9	0.05	10
10	8.6 \pm 4.3	356.2 \pm 64.9	-8.04	7	0.001	8
100	8.0 \pm 8.0	235.1 \pm 43.1	-8.90	9	0.0005	10

* Paired *t*-test.**Fig. 2. Mean \pm SEM number of *H. axyridis* adults in test (DEET) vs control (hexane blank) arm of a 3-neck bulb-tube olfactometer per 5-min time interval in 32 observations.**

treated with all three concentrations of DEET than the control (acetone blank) in this 60-min bioassay in which honey was present at the center of each 42.5-mm disk (Fig. 3A). At 5 d after application, significantly more beetles avoided disks treated with 0.1 and 1.0 mg/cm² of DEET rather than 0.01 mg/cm² or the control. Note that the number of beetles in control disks was significantly different between 1 d and 5 d after application. The hunger level of beetles used on 1 d and 5 d was not standardized. Although not presented in Fig. 3, the avoidance response of males (in trial 1) did not differ from females (trial 2) in this experiment ($F = 0.3$; $df = 1, 87$; $P = 0.6$); so data were pooled.

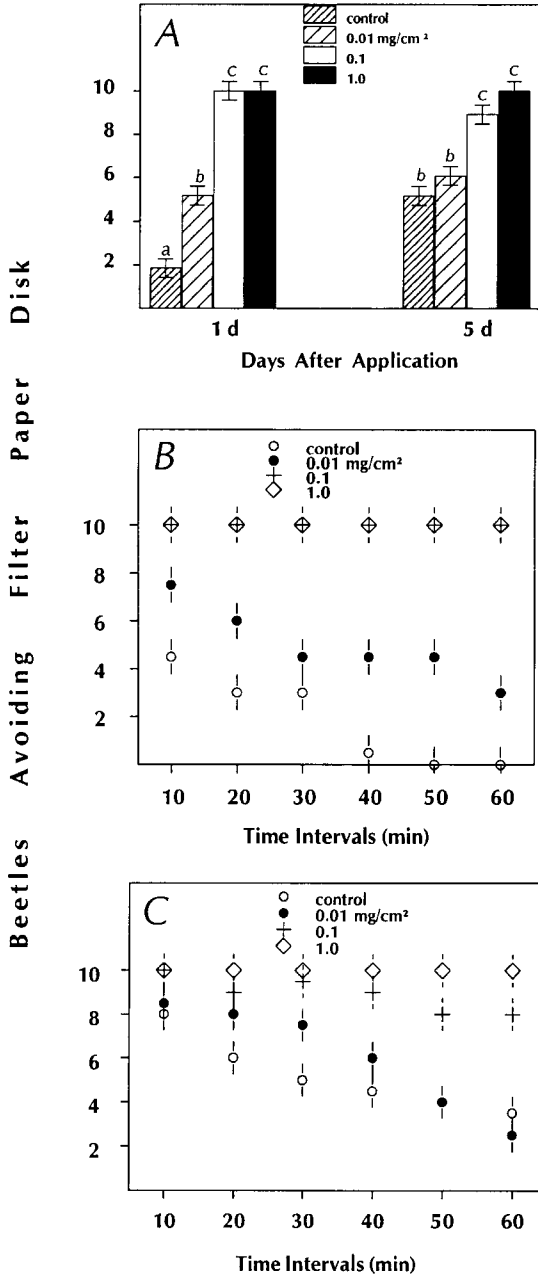


Fig. 3. Least squares mean \pm SEM number of *H. axyridis* adults avoiding filter paper disks treated with DEET (0.01, 0.1 and 1.0 mg/cm²) at 1 d and 5 d after application, summarized (A), and at 1 d after (B) and 5 d after application (C) per 10-min time interval in 96 observations.

The avoidance response of beetles at each 10-min time interval is illustrated (Fig. 3B, C). At 1 d after application, all beetles avoided disks treated with 0.1 and 1.0 mg/cm² of DEET (Fig. 3B); whereas, the avoidance of disks treated with 0.01 mg/cm² of DEET or the control declined gradually over time. At 5 d after application, all beetles avoided the 1.0 mg/cm² concentration, but there was a slight decline in avoidance of disks treated with 0.1 mg/cm². A more pronounced decrease in avoidance was evident with 0.01 mg/cm² of DEET.

Bioassay in Petri dish with strip. The Petri dish with strip bioassay indicated that the interaction between day after application and DEET or camphor concentration was significant ($F = 5.0$; $df = 6, 28$; $P = 0.001$; Fig. 4). Effects of day and concentration were significant when analyzed separately (day: $F = 36.8$; $df = 1, 28$; $P < 0.0001$; concentration: $F = 52.8$; $df = 6, 28$; $P < 0.0001$). At 1 d after application, all concentrations of D-P (DEET-paraffin mix) and 1% and 9% concentrations of C-P (camphor-paraffin mix) elicited avoidance responses in beetles that were significantly greater than the control (paraffin alone). At 23 d after application, only the 1% and 9% D-P concentrations elicited responses greater than the control (Fig. 4).

Avoidance behaviors included jumping back from the edge of the treated paper strip and turning away from the edge. Jumping back from the treated strip was interpreted as an indication of high repellency; turning away from the strip indicated moderate repellency; and crossing over the strip indicated no demonstrable repellency. At 1 d after application, 86.7 and 13.3% of beetles exposed to 9% D-P jumped back or turned away, respectively ($n = 30$). Some 53.3 and 33.3% of those exposed to 1% D-P jumped back or turned away, respectively ($n = 30$). The response of

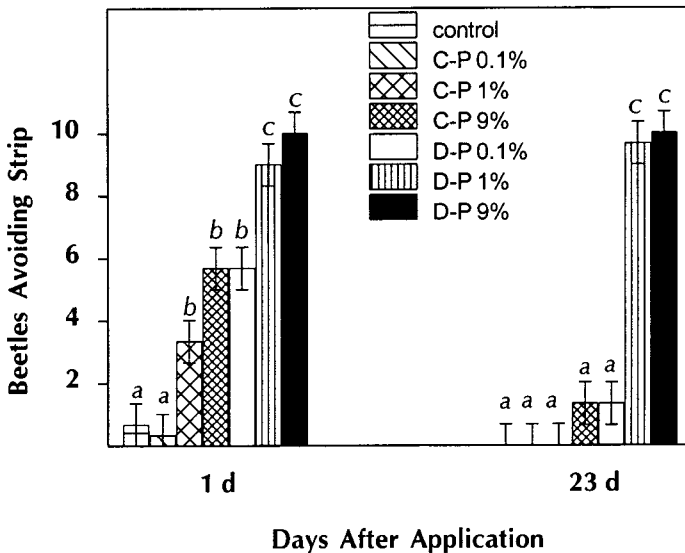


Fig. 4. Least squares mean \pm SEM number of *H. axyridis* adults avoiding filter paper strip treated with a formulation of DEET or camphor and paraffin wax (~ 0.1, 1 and 9% mixtures) at 1 d and 23 d after application in 42 observations.

beetles to camphor was less pronounced; 3.3 and 50% of beetles jumped back or turned away, respectively, when exposed to strips coated with 9% C-P ($n = 30$). No beetles jumped back and 33.3% turned away from strips coated with 1% C-P ($n = 30$) 1 d after application.

At 23 d after application, 70 and 30% of beetles exposed to strips coated with 9% D-P jumped back and turned away, respectively ($n = 30$); 40% jumped back and 56.7% turned away when exposed to 1% D-P ($n = 30$). Neither the 9% nor the 1% C-P had any demonstrable repellency against beetles relative to the control 23 d after application.

Discussion

The discovery that DEET elicited avoidance behavior in *H. axyridis* adults in the bulb-tube and γ -tube olfactometer bioassays indicates that volatiles were detected by olfactory receptors. As far as is known, antennae and, to a lesser extent, maxillary palpi are involved in chemoreception in aphidophagous coccinellids (Barbier et al. 1989, Jourdan et al. 1995, Hamilton et al. 1999, Zhu et al. 1999, Al Abassi et al. 2000). The precise mechanism responsible for triggering an avoidance response to DEET is unknown for lady beetles, or any other coleopterans. Perhaps, this compound simply irritated the chemoreceptors on antennae and palpi of *H. axyridis* adults.

Dogan et al. (1999) and Hoffman and Miller (2002) indicated that both the vapor and liquid phases of DEET are effective against mosquitoes. DEET may inhibit lactic acid receptors on antennae of certain female mosquitoes; if lactic acid (which is a component of human sweat) is not present, DEET can potentially attract mosquitoes (Dogan et al. 1999).

In this study, the fact that *H. axyridis* adults avoided the side arm containing 142 μg of DEET in the bulb-tube bioassay suggests that this compound performs as well as camphor. Previous research using the same olfactometer design indicated that 142 μg of R-(+)-camphor elicited a similar avoidance response (Riddick et al. 2000). Although behavioral responses to various concentrations of DEET could not be directly compared (in the γ -tube bioassay), the data suggest decreasing avoidance of the side arm containing DEET at lower concentrations. In the γ -tube bioassay, 10 μg of DEET was shown to elicit an avoidance response in beetles. However, 10 μg of camphor or menthol did not elicit a significant avoidance response in a previous study using the same type of olfactometer (Riddick et al. 2000). The results of the olfactometer bioassays with DEET suggest that this compound could be more repellent than camphor or menthol for *H. axyridis* adults.

The results of the Petri dish with disk bioassay (using food as an attractant) indicated that DEET had some residual activity and that the presence of food (i.e., honey) was not sufficiently attractive to induce beetles to spend time on disks treated with 0.1 or 1.0 mg/cm^2 of DEET. The fact that more than 80% of beetles avoided disks treated with DEET at these two rates at 5 d after application was unexpected and suggests that DEET has some residual activity. Control disks were freshly treated with acetone on the fifth day after applying DEET to the test disks, which may account for the observation that more beetles avoided control disks on 5 d rather than 1 d after application (see Fig. 3A). This could also explain why there was no significant difference in avoidance behavior for beetles exposed to the control vs 0.01 mg/cm^2 of DEET 5 d after application.

To our knowledge, only two previous studies have tested the potential of DEET to

modify the behavior of coleopterans and both concern stored-product pests. Watson and Barson (1996) demonstrated that DEET elicited avoidance behavior of adults of three strains of the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.), within 7 h of exposure to filter paper treated with this compound, at a concentration of 250 mg/m², in Petri dish arenas. In a similar study that involved the use of a computerized tracking system, DEET (250 mg/m²) was found to elicit an avoidance response in adults, but not larvae, of the varied carpet beetle, *Anthrenus verbasci* (L.), in Petri dish arenas (Watson et al. 1997). Adults spent more time and moved more rapidly on the untreated side than on the treated side of the filter paper; adults turned back and remained on the untreated side whenever they approached the boundary of the treated paper (Watson et al. 1997).

The results of the Petri dish with strip bioassay (using DEET or camphor mixed with melted paraffin) suggest that DEET has considerably more residual activity than camphor and that a 1% DEET/paraffin mixture has the potential to persist for 3 wks. The fact that a 1% mixture of DEET/paraffin was as effective as a 9% mixture suggests that only a small quantity of this compound will be necessary. This experiment revealed that *H. axyridis* adults do not have to make physical contact with DEET-treated strips (at 1% and 9% concentrations) in order to detect DEET molecules and respond to them. Many of the beetles were observed jumping back or turning away from coated strips before antennae or palpi made contact with the strip. Apparently, paraffin reduced the rate of vaporization of DEET molecules, such that molecules remained in the paraffin for more than 23 d after application at the two highest rates.

Differences in vapor pressure between DEET and camphor may partly explain the differences in repellency between them in the Petri dish with strip bioassay. The vapor pressure of DEET is 0.002 mm Hg at 25°C; whereas, the vapor pressure of camphor is 0.26 mm Hg at the same temperature. If vapor pressure is directly proportional to evaporation rate, DEET would likely evaporate from paraffin more slowly than camphor, because DEET has a much lower vapor pressure. Also, the molecular weight of DEET (191.3) is greater than that of camphor (152.2). This suggests that more energy is required to displace molecules of DEET than of camphor under similar conditions of solvation.

Despite the apparent effectiveness of DEET, it has some drawbacks including potential health risks at high dosages (Qui et al. 1998) and the potential for damaging plastics that come in contact with it. Thus, protective clothing must be worn when applying a DEET-based product on the house exterior, and DEET should only be applied to unpainted or non-plastic surfaces, such as wood or brick. Some alternative compounds that purportedly do not dissolve plastics (e.g., certain piperidine analogs) have been shown to repel mosquitoes as effectively as DEET (Klun et al. 2003), and might also repel multicolored Asian lady beetles from house exteriors.

Conclusion. This study demonstrates the ability of DEET to modify the behavior of *H. axyridis* adults in the laboratory. Follow-up field studies will be necessary to validate these laboratory results in the urban environment. A 'DEET strip' might be placed around window frames, ventilation openings, and other suspected entry points on home exteriors to discourage beetle entry. Alternatively, a spray formulation of DEET might be targeted directly onto cracks or crevices not accessible from the ground. A spray formulation must be capable of sustained repellency for 1 to 2 wks to be useful to homeowners.

Research leading to the identification of chemical attractants and an efficient outdoor trap is necessary before a push-pull strategy of managing *H. axyridis* can be

come a reality. Pushing lady beetles away from houses with repellents and pulling them into traps baited with attractants is a bio-rational approach to solving this nuisance problem. Trapped beetles could be cold-stored in depositories until the spring, then released to serve as predators of plant pests.

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