# Potential for Modifying the Behavior of the Multicolored Asian Lady Beetle (Coleoptera: Coccinellidae) with Plant-Derived Natural Products

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**ABSTRACT** Bioassays were conducted to study the effectiveness of selected chemicals to prevent the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), from overwintering in buildings. We discovered that certain monoterpenoids elicited avoidance in adults toward treated filter paper within a petri dish bioassay at 1.0 mg/cm$^2$. Camphor and menthol were the most effective of the monoterpenoids tested. Y-tube olfactometer bioassays revealed that beetles spent significantly more time (within 10-min observation periods) in the untreated control arm than in the arm containing camphor or menthol (both at 100 and 1,000 $\mu$g). Another olfactometer bioassay revealed that significantly more beetles remained in the untreated control arm than in the arm containing camphor or menthol (142 $\mu$g), within 45-min observation periods. When camphor (9.4% emulsified concentrate) was sprayed onto 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, two replicates). In another field experiment, significantly fewer *H. axyridis* were captured in traps containing camphor versus un-baited control traps. Research is continuing to develop a protocol for repelling nuisance beetle aggregations and conserving the beetles for biological control applications.

**KEY WORDS** lady beetles, *Harmonia axyridis*, repellents, natural products, camphor, push-pull concept

DESPITE NUMEROUS ATTEMPTS to introduce the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas), into the United States it did not become firmly established until 1988 (Chapin and Brou 1991). It was released during 1978–1981 to control aphids in pecan orchards in the Southeast (Tedders and Schaefer 1994). Currently, *H. axyridis* is abundant throughout the United States, especially in the northeastern states and eastern Canada (Day et al. 1994, Hoebeke and Wheeler 1996).

In the spring and summer seasons, *H. axyridis* larvae and adults are important predators of aphids and scale insects (Kidd et al. 1995, La Mana and Miller 1996, Brown and Miller 1998). In the fall, adults migrate from feeding sites to seek shelter for overwintering. For example, in Kyoto, Japan, adults were frequently found aggregating within the cracks and crevices of rock outcroppings, but aggregations were also found inside a wooden hut. Beetles appeared to orient toward light-colored objects within these sites (Obata 1986). In Honshu, Japan, beetles were aggregated in dark places inside whitish-colored buildings (Sakurai et al. 1993). In the United States, adults also form aggregations for overwintering. In recent years, the most conspicuous sites have been inside sheltered places in buildings and houses (Kidd et al. 1995, Nalepa et al. 1996). The propensity of adults to enter houses in the fall season has become a serious concern to homeowners. Beetles that successfully enter houses eventually aggregate by the thousands in secluded dark places (commonly attics). On warm spells during winter and spring, beetles often become active and again become a nuisance in houses.

There is an urgent need to discover how to safely thwart the entry of these otherwise desirable lady beetles into buildings. Currently, there are no techniques for keeping beetles away from dwellings without killing them. At this point, some homeowners and professional exterminators resort to spraying broad-spectrum insecticides on the exterior of homes. There are no chemicals designed to specifically repel *H. axyridis* or any other beneficial lady beetle from buildings.

Here we considered the potential of monoterpenoids as repellents to prevent *H. axyridis* from entering human dwellings. Monoterpenoids are secondary plant compounds, consisting of a 10-carbon, isoprene-derived skeleton. They are thought to act as a means of chemical defense against some phytophagous insects, bacteria, and fungi (Whittaker 1970). In recent years, a number of monoterpenoids have been considered as alternatives to conventional insecticides.

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Several have shown toxicity or repellency to insects (Harwood et al. 1990; Cook 1992; Coats 1994; Rice and Coats 1994a, 1994b; Cornelius et al. 1997), and most are not acutely toxic to mammals (Coats 1994). Many are considered safe and have been used as artificial flavorings in foods, in the production of perfumes (Templeton 1969), and for medicinal and antiseptic purposes (Klocke et al. 1987). For example, camphor was shown to effectively kill the bean weevils *Callosobruchus chinensis* (L.) inside air-tight containers (Abivardi 1977, Abivardi and Rahimian 1977). More recently, camphor, in combination with certain plant oils, effectively protected stored grain from beetle pests (Obeng-Ofori and Reichmuth 1999). Camphor also has a long history of use to protect clothing from household insects (Abivardi and Benz 1984, Chauvin and Vannier 1994). Menthol has been used in honey bee hives to control parasitic mite infestations (Cox et al. 1989, Westcott and Winston 1999), and it has been used as a repellent to disperse aggressive honey bees away from humans (Collins et al. 1996).

Our objective was to evaluate the effectiveness of selected chemicals, some of which are known repellents against biting insects, to modify the behavior of *H. axyridis* adults.

## Materials and Methods

**Source of Beetles for Laboratory Experiments.** Adult *H. axyridis* were collected from overwintering aggregations inside a watchtower near Lancaster, PA, in December 1996. Approximately 5,000 beetles were brushed from the inner walls of this structure into plastic containers and stored in ice chests for transport to the laboratory.

**Maintaining Beetles in the Laboratory.** Beetles were maintained in clear plastic, 3.8-liter cookie containers with screened lids: ≈200 beetles per container, with crumpled paper towels, cotton-stoppered water vials, and honey smeared on the screened lids. Beetles were maintained in an incubator at 15°C and a photoperiod of 12:12 (L:D) h; once a week they were moved to room temperature for 4–8 h, fed honey, and misted with water. Before behavioral testing, beetles were placed at room temperature for 1–2 h. At this time beetles were once again misted with water and fed.

**Bioassays in Petri Dish Arenas.** The chemicals tested included *R*-(+)-limonene, *a R*-(+)-camphor, *R*-(-) carvone, *S*-(+)-carvone, (±)-menthol, geraniol, eugenol, citronellol, 1, 8-cineole, citral, and citronellal. Hexane was included as a control. Chemicals were purchased from Aldrich (Milwaukee, WI), with the exception of citronellal, which was purchased from Givaudan Roure (Cincinnati, OH). Dilutions of each test chemical were prepared in HPLC-grade hexane, such that a volume (129 μl) needed to wet the entire area of a 1 by 9-cm strip of filter paper would result in a known application rate (e.g., 9 mg/129 μl results in a 1 mg/cm$^2$ application rate applied to a strip). We tested two concentrations, 0.001 and 1.0 mg/cm$^2$. The treated strip was positioned and taped (underneath) to the edge of a one-half circle of filter paper. The solvent evaporated before the strip was taped to the untreated filter paper. Each petri dish was placed horizontally under a chemical flow hood (with bright fluorescent lights, 23 ± 2.5°C) before adding a beetle. Each beetle was gently placed in the glass petri dish (9 cm diameter, 1.5 cm deep), without the lid, on the untreated one-half circle and faced in the direction of the treated strip. A series of 10 beetles were subjected to the treatments, one at a time. The response of each beetle was determined within 10 s after it approached the treated strip. The direction of each beetle inside each dish was alternated between facing the back or the front of the hood after each series. Beetles that flew out of the dish before testing were not used. There were from 3 to 6 series of 10 beetles tested per concentration per chemical. Each beetle was tested only once. The test strip was replaced for each beetle tested. A separate, clean petri dish was used for each beetle. Afterward, each petri dish was thoroughly washed in soapy water and air dried. Experiments were conducted between 1000 and 1700 hours.

**Bioassays in Y-Tube Olfactometer.** A glass Y-tube olfactometer (≈5 cm arms, 1 cm i.d.) was used to compare the amount of time that individual beetles spent in the test versus control arm in response to volatiles. A strip of treated filter paper (2 by 0.5 cm) was held 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, Louis, MO) and humidified through a Dudley bubbling tube before entering test or control arms of the Y-tube olfactometer. All components were connected by lengths of silicon tubing, and the flow rate exiting the olfactometer was adjusted to 100 ml/min with a calibrated soap-bubble air flow meter and stopwatch. The apparatus was positioned horizontally on a countertop in the laboratory (at 23 ± 2.5°C) with bright fluorescent lights. Experiments were conducted between 1000 and 1700 hours.

Ten microliters of the test or control chemicals were used on the filter paper strip. Test solutions were camphor or menthol at 1 μg/μl, 10 μg/μl, and 100 μg/μl in hexane. The control was a hexane blank. After the chemicals were applied to the filter paper and placed inside the Y-tube, a beetle was added to the base of the apparatus by removing the cotton plug. The plug was repositioned and the air flow was restored through the apparatus. The amount of time (in seconds) that each beetle spent in the test versus the control arm during a 10-min period was recorded. Each test was repeated 10–20 times per dilution per chemical. Test and control arms (sides) of the olfactometer were alternated after every trial. We used four Y-tube olfactometers (all of approximately the same dimensions) interchangeably. Each olfactometer was washed in soapy water and dried at ≥100°C between trials.

**Bioassays in Bulb-Tube Olfactometer.** A glass bulb-tube olfactometer (Borges and Aldrich 1994) was used to compare the number of beetles that moved into test

versus control arms in response to volatiles. Side arms were two splash-guard adapters (250 ml) connected at their lower joints to a round-bottom, 3-angled neck distilling flask (100 ml). The central neck of the distilling flask was fitted with an adapter connected to the house vacuum. Ambient air was drawn by vacuum through a Dudley bubbling tube (a humidifier), through a glass filter tube containing charcoal and glass wool at the outer joint of each arm, through the distilling flask, and finally through the central neck. All glassware was connected using silicon tubing. The air flow rate was set at 100 ml/min through both test and control side arms. The apparatus was positioned horizontally on a countertop in the laboratory (at 23 ± 2.5°C) with bright fluorescent lights. Experiments were conducted between 1000 and 1700 hours.

Two microliters of test or control chemicals were applied to a disk of filter paper (2.1 cm diameter). The disks were placed in test or control arms. Test solutions were camphor or menthol at 71 $\mu$g/$\mu$l in hexane. The control was a hexane blank. At the same time, 12 beetles were placed into the central area of the round-bottom flask through the central neck. The adapter attached to the central neck was rejoined and the vacuum restored to the apparatus. We began timing and observing beetle behavior as soon as the vacuum was restored. Beetles easily traversed the olfactometer by crawling on the inner walls of the glassware. The number of beetles present in test and control arms was recorded at 5-min intervals, up to 45 min. Test and control arms (sides) were alternated after every trial. Each test was repeated five times. We used two bulb-tube olfactometers (all of the same dimensions) interchangeably. Each olfactometer was washed in soapy water then dried at ≥100°C between trials.

**Bioassays at the Field Site in Beltsville, MD.** Beetles flying and landing on the brick walls of the National Agricultural Library (NAL), Beltsville, MD, were first observed in October 1995 and again in October 1996. Experimentation at this site began in October 1997. During this month three crevices were detected through which beetles were entering and moving behind the outer wall of the NAL. The beetles were most active and often seen entering the three particular crevices in the NAL wall in the afternoon on mostly sunny days. All crevices were on the side of the building exposed to the afternoon sun.

An emulsifiable concentrate of menthol or camphor (9.4% [AI]) containing menthol or camphor (9.6 g), xylene (4.0 ml), 1-octyl-2-pyrrolidinone (400 $\mu$l), Tween 80 (8.0 ml, at 10 $\mu$g/$\mu$l in hexane), and distilled water (80 ml) was prepared (Inert ingredients were purchased from Aldrich). A spray bottle (≈60 ml) was used to apply the emulsion. After priming, two complete pumps of the nozzle delivered ≈200 $\mu$l of concentrate. Sprays were applied on 8, 10, 29, and 31 October 1997 at NAL. Two of the three crevices that we discovered were used in this experiment. Crevice #1 was located on the second floor roof of the NAL; crevice #2 was located on the first level of the building. Crevice #1 was essentially a small opening (1.3 by 3.8 cm) that was, inadvertently, left unplastered. Crevice #2 was a vertical slit (≈6 by 1.5 cm) resulting from the breaking-away of the sealant or mortar. Both crevices were exposed to afternoon sun. All beetles crawling near both crevices were gently removed from the substrate by trapping inside a plastic vial or by using an aspirator. When all beetles had been removed, the test concentrate (menthol or camphor) was sprayed directly onto the crevice and its periphery. The number of beetles that approached the treated crevice and the area peripheral to the crevice was recorded. The number of beetles that traversed the treated substrate and entered the crevice was also recorded.

**Bioassays at the Field Site in Monticello, FL.** Another field experiment was conducted to determine if camphor would repel beetles from entering insect traps in the field. For this experiment the camphor solution was prepared by dissolving 2 g of crystalline camphor in 5 ml of acetone to produce a 400 $\mu$g/$\mu$l solution of camphor. We applied 100 $\mu$l of this solution to 2-cm sections of a cellulose (cigarette) filter and allowed the acetone to evaporate before placing the filter inside a 1-ml centrifuge tube with cap. Essentially, the tube contained 40 mg of active ingredient. We filled a series of 40 centrifuge tubes with the treated cellulose filter on the same day. Each tube had a tight-fitting snap-on cap that prevented evaporation of the active ingredient. Tubes were stored in a refrigerator before being used in the experiment.

The trap design at this location involved the use of a Rescue! Yellowjacket trap (Sterling, Liberty Lake, WA) mounted on top of a tin funnel. A wire screen was placed underneath the funnel to make it easier for the beetles to walk up into the funnel and then into the yellowjacket trap. Tin foil was placed around the exterior of the yellowjacket trap; this made the interior of the yellowjacket trap appear dark. The yellowjacket trap with funnel was mounted at the apex of a white Chloroplast panel (0.6 m wide by 2.4 m tall, PBE Graphics Warehouse, West Palm Beach, FL). The panel was held upright and anchored in the ground with a wooden post (≈5 cm diameter) at the back of the panel. The base of the panel was flush with the soil surface. Thus, flying or crawling beetles were able to reach the panel and crawl upward toward the apex. This trap design was used at two locations in or near Monticello, FL, in November and December 1997. In the two field sites (a pecan orchard and a house), one test trap and one control trap were deployed. One camphor-containing centrifuge tube (with cap removed) was mounted inside each test trap. The control traps contained no tube and were devoid of repellent chemical. Traps were checked for beetles on 11, 12, 14, 21, 24, and 26 November, and on 1 and 4 December. At each collection date, the tube containing camphor was replaced with an unused tube of the same chemical. Captured beetles were removed from the test or control traps, counted, returned to the laboratory, and held in cold storage.

**Statistical Analyses.** The mean number of beetles avoiding or crossing the filter paper strip in the petri dish arenas was compared using the Student *t*-test, when data met the assumptions of normality, or the

**Table 1. Mean ± SEM number of beetles avoiding or crossing filter paper strip treated with test chemicals in petri dish arenas**

| Chemical | Concn (mg/cm$^2$) | Avoid | Cross | t or T[a] | df | *P* | *n* |
|---|---|---|---|---|---|---|---|
| Hexane (solvent) | — | 0.00 ± 0.00 | 10.0 ± 0.00 | — | — | — | 12 |
| R-(+)-limonene | 0.001 | 1.67 ± 0.71 | 8.33 ± 0.71 | −6.6 | 10 | < 0.01 | 12 |
| | 1.0 | 6.33 ± 0.33 | 3.67 ± 0.33 | 5.7 | 10 | < 0.01 | 12 |
| R-(+)-camphor | 0.001 | 3.67 ± 0.71 | 6.33 ± 0.76 | −2.9 | 10 | 0.02 | 12 |
| | 1.0 | 9.83 ± 0.17 | 0.17 ± 0.17 | 57.0[a] | — | < 0.01 | 12 |
| R-(−)-carvone | 0.001 | 2.50 ± 1.12 | 7.50 ± 1.12 | 25.5[a] | — | 0.03 | 12 |
| | 1.0 | 8.50 ± 0.67 | 1.50 ± 0.67 | 7.4 | 10 | < 0.01 | 12 |
| S-(+)-carvone | 0.001 | 1.83 ± 1.05 | 8.17 ± 1.05 | −4.3 | 10 | < 0.01 | 12 |
| | 1.0 | 7.00 ± 0.63 | 3.00 ± 0.63 | 4.5 | 10 | < 0.01 | 12 |
| (±)-menthol | 0.001 | 2.17 ± 0.48 | 7.83 ± 0.48 | −8.4 | 10 | <0.01 | 12 |
| | 1.0 | 7.33 ± 0.76 | 2.67 ± 0.76 | 4.3 | 10 | < 0.01 | 12 |
| Geraniol | 0.001 | 1.83 ± 0.98 | 8.17 ± 0.98 | −4.6 | 10 | < 0.01 | 12 |
| | 1.0 | 8.83 ± 0.48 | 1.17 ± 0.48 | 11.4 | 10 | < 0.01 | 12 |
| Eugenol | 0.001 | 0.33 ± 0.21 | 9.67 ± 0.21 | −31.3 | 10 | < 0.01 | 12 |
| | 1.0 | 9.17 ± 0.48 | 0.83 ± 0.48 | 12.3 | 10 | < 0.01 | 12 |
| Citronellol | 0.001 | 0.50 ± 0.34 | 9.50 ± 0.34 | −18.6 | 10 | < 0.01 | 12 |
| | 1.0 | 8.00 ± 0.36 | 2.00 ± 0.36 | 11.6 | 10 | < 0.01 | 12 |
| 1, 8-cineole | 0.001 | 0.33 ± 0.21 | 9.67 ± 0.21 | −31.3 | 10 | < 0.01 | 12 |
| | 1.0 | 5.50 ± 0.43 | 4.50 ± 0.43 | 1.6 | 10 | 0.13 | 12 |
| Citral | 0.001 | 2.00 ± 1.15 | 8.00 ± 1.15 | −3.7 | 4 | 0.02 | 6 |
| | 1.0 | 8.00 ± 1.00 | 2.00 ± 1.00 | 4.2 | 4 | 0.01 | 6 |
| Citronellal | 0.001 | 4.33 ± 0.88 | 5.66 ± 0.88 | −1.1 | 4 | 0.34 | 6 |
| | 1.0 | 6.33 ± 1.20 | 3.66 ± 1.20 | 1.6 | 4 | 0.19 | 6 |

[a] *T*, statistic for the Mann-Whitney test. $P \leq 0.05$, significant difference detected between avoid and cross behaviors. See *Materials and Methods* for details.

Mann–Whitney rank sum test when they did not (Glantz 1992, Forthofer and Lee 1995). The mean amount of time that beetles spent in test versus control arms in the Y-tube olfactometer was compared using the paired *t*-test or the Wilcoxon signed-rank test, if data were not normally distributed. The mean number of beetles present in the test versus control arms of the bulb-tube olfactometer was compared using the paired *t*-test. The Pearson product-moment correlation coefficient or the Spearman rank correlation coefficient (if data were not normally distributed) was used to recognize any association between the occurrence of beetles in the test versus control arm during consecutive 5-min time intervals in the bulb-tube olfactometer. Data from the field experiment in Beltsville, MD, were not readily amenable to statistical analysis; these data were tabulated only. The mean number of beetles captured in test versus control traps in or near Monticello, FL, was compared using the Student *t*-test. Statistical analyses were performed with Sigma Stat software (Sigma Stat 1994). For all experimental analyses, means were considered significantly different if $P \leq 0.05$.

## Results

**Behavioral Responses of Beetles in Petri Dish Arenas.** For all but two of the test chemicals, significant differences were detected between the mean number of beetles avoiding the treated paper strip at the high (1.0 mg/cm$^2$) but not the low (0.001 mg/cm$^2$) concentration (Table 1). Avoidance behaviors included jumping back from the edge of the treated paper or turning away from the edge. At the high concentration, adults were often repelled; at the low concentration, adults usually crossed the treated paper without hesitation. When comparing the avoidance response of the beetles at the high concentration among the range of chemicals, camphor displayed the highest repellency; 98% avoided the treated strip. The second highest response was elicited by eugenol, 91.7% avoided the strip at the high concentration. When comparing the avoidance response toward these same chemicals, at the low concentration, camphor elicited the greatest repellency. Menthol and *R*-carvone elicited relatively strong avoidance responses in the beetles at the high concentration, while maintaining some repellency at the low concentration. No avoidance response was elicited by 1, 8-cineole and citronellal at the high concentration. Also, there was no significant difference between avoiding or crossing the strip treated with citronellal at the low concentration.

**Behavioral Responses of Beetles in Y-Tube Olfactometer.** At the low concentration (10 $\mu$g), there was no significant difference in the amount of time that each beetle spent within the test arm containing camphor versus the control arm (Fig. 1A, paired *t*-test, $t = -0.64$, df = 9, $P = 0.53$, $n = 10$ per arm). At the moderate concentration (100 $\mu$g), significantly less time was spent in the test arm than in the control arm ($t = -5.4$, df = 9, $P < 0.01$, $n = 10$ per arm). At the highest concentration, almost all time was spent in the control arm ($t = -8.8$, df = 9, $P < 0.01$, $n = 10$ per arm), instead of the test arm containing camphor.

When menthol was used, beetles did not display a significant avoidance of this chemical in comparison to the control at the low concentration (Fig. 1B, paired *t*-test, $t = 0.95$, df = 9, $P = 0.36$, $n = 10$ per arm). But at the moderate concentration, beetles were repelled

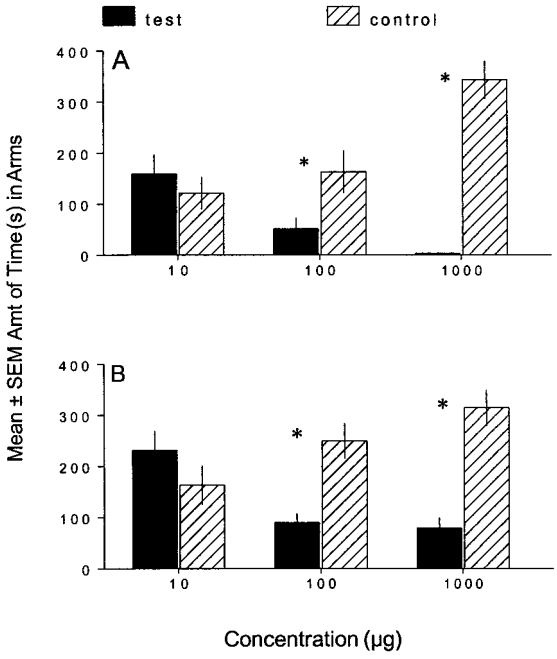


**Fig. 1.** Mean ± SEM amount of time (seconds) spent by beetles in test versus control arm in Y-tube olfactometer. (A) Test arm contained camphor and the control arm contained hexane. (B) Test arm contained menthol and the control arm contained hexane. *, statistically significant difference between treatments. For camphor exp, *n*, 10 observations per arm at 10, 100, and 1,000 µg. For menthol exp, *n*, 10 observations per arm at 10 and 1,000 µg, and 20 observations per arm at 100 µg.

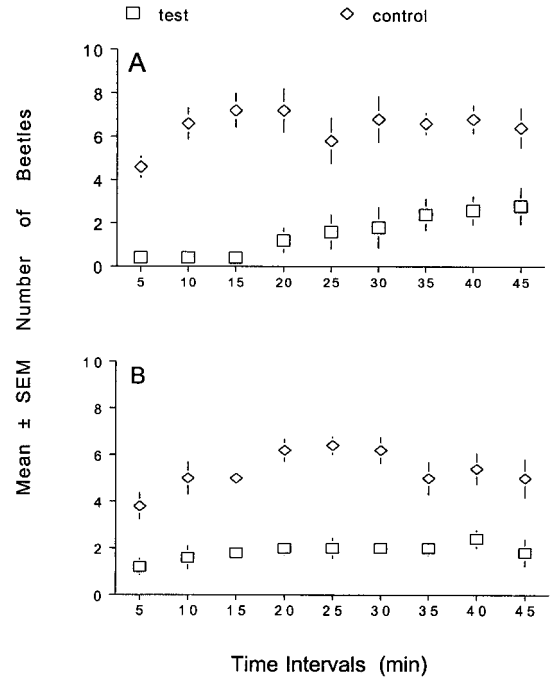


**Fig. 2.** Mean ± SEM number of beetles present within test arm versus control arm per time interval in Bulb-tube olfactometer. (A) Test arm contained camphor and the control arm contained hexane. (B) Test arm contained menthol and the control arm contained hexane. *, statistically significant difference between treatments. For camphor and menthol exp, *n*, 45 observations per arm.

by menthol ($t = 3.5$, df = 19, $P < 0.01$, $n = 20$ per arm). At the high concentration, significantly fewer beetles spent time in the test arm than in the control arm ($t = -4.6$, df = 9, $P < 0.01$, $n = 10$ per arm).

**Behavioral Responses of Beetles in Bulb-Tube Olfactometer.** Fig. 2A summarizes the response of the group to camphor (in test arm) versus the hexane blank (in control arm) at 5-min intervals. At 5-, 10-, and 15-min intervals only 0.4 ± 0.2 (mean ± SEM) beetles had moved into the test arm and 4.6 ± 0.5 beetles had moved into the control arm. By the 25-min interval, 1.6 ± 0.8 beetles were in the test arm and 5.8 ± 1.1 beetles were in the control arm. By the 45-min (and final) interval, 2.8 ± 0.9 beetles were in the test arm, 6.4 ± 0.9 beetles were in the control arm. There was a weak negative association between the paired responses of beetles, in the test arm versus the control arm, in this experiment (Pearson product-moment correlation, $r = -0.47$, $P < 0.01$, $n = 45$). As the number of beetles in the test arm increased, the number of beetles in the control arm decreased. The overall number of beetles per interval was 1.5 ± 0.2 in the test arm (camphor) and 6.4 ± 0.3 in the control arm (hexane blank). There was a statistically significant difference between the two treatments; fewer beetles occupied the arm containing the camphor solution (paired *t*-test, $t = -10.3$, df = 44, $P < 0.01$, $n = 45$ per arm).

Figure 2B illustrates the responses of a group of beetles to menthol at 5-min intervals. At 5 min, 1.2 ± 0.4 beetles were present in the test arm, but 3.8 ± 0.6 beetles were in the control arm. At the 25-min interval, 2.0 ± 0.45 beetles were in the test arm and 6.4 ± 0.4 were in the control arm. At the 45-min interval, 1.8 ± 0.6 beetles were in the test arm and 5.0 ± 0.8 were in the control arm. The greatest number of beetles ever present in the test arm was 2.4 ± 0.4, as evidenced at the 40-min interval. There was no significant association between the paired responses (test arm versus control arm) of beetles in this experiment (Pearson product-moment correlation, $r = 0.17$, $P = 0.26$, $n = 45$). There was no significant increase in the number of beetles moving into the test arm during this experiment. The overall number of beetles per interval in the test arm (menthol) was 1.9 ± 0.1, but 5.3 ± 0.2 beetles in the control arm (hexane blank). Significantly fewer beetles were found in the test arm than in the control arm (paired *t*-test, $t = -14.1$, df = 44, $P < 0.01$, $n = 45$ per arm).

**Behavioral Responses of Beetles in The Field—Beltsville, MD.** On 8 October 1997, observations were made at crevice #1 and its periphery. The air temperature in Beltsville (USDA facility, weather station #2) was a maximum of 27.6°C and a minimum of

**Table 2. Percentage of *H. axyridis* repelled from entering crevices at NAL, Beltsville, MD**

| Concn 9.4% | Amt, μl | % repelled | *n* | Time, h | Date | Crevice |
|---|---|---|---|---|---|---|
| Menthol | 200 | 66.6 | 6 | 0.25 | 08/10/1997 | #1 |
| Camphor | 800 | 100 | 19 | 0.50 | 10/10/1997 | #1 |
| Menthol | 800 | 75.0 | 8 | 0.33 | 29/10/1997 | #2 |
| Camphor | 800 | 100 | 27 | 0.50 | 31/10/1997 | #2 |

13.5°C. The skies were mostly sunny and the crevice was fully exposed to afternoon sun. A menthol concentrate was sprayed on crevice #1 and its periphery and observations were made between 1555 and 1610 hours. During this 0.25-h period, 66.6% of the approaching beetles were repelled (Table 2). The repelled beetles came within ≈5 cm of the treated substrate and stopped crawling, as if detecting the odor of menthol. In response, they generally backed away or flew away from the treated substrate. Other repelled beetles backed away, then tried to enter the crevice from a different direction. Because the menthol concentrate was sprayed on and around the crevice, these beetles were not able to enter, because there was no untreated passageway leading into the crevice. The two beetles that did enter the crevice did so by traversing the treated substrate, appearing agitated but not deterred.

On 10 October 1997, further observations were made at crevice #1. The air temperature in Beltsville was a maximum of 27.9°C and a minimum of 14.7°C. The skies were mostly sunny and crevice #1 was fully exposed to the sunlight. Beetles were seen entering this crevice on this afternoon without any hesitation—thus the menthol that was sprayed previously on this crevice (see previous paragraph) had dissipated. A camphor concentrate was applied to crevice #1. Observations were made between 1530 and 1600 hours. During this 0.50-h period, 100% of the approaching beetles appeared to be repelled from entering the treated crevice (Table 2). These beetles either backed away or flew away from the treated substrate.

On 29 October 1997, observations were made at crevice #2. The air temperature in Beltsville was a maximum of 15.6°C and a minimum of −1.4°C. The skies were mostly sunny and crevice #2 was fully exposed to sunlight. The menthol concentrate was sprayed on this crevice. Observations were made between 1554 and 1615 hours. During this 0.33-h period, 75% of the approaching beetles were repelled from entering the treated crevice (Table 2).

On 31 October 1997, observations were also made at crevice #2. Beetles were entering the crevice without hesitation, at a rate of ≈1 beetle per 45–60 s. The air temperature in Beltsville was a maximum of 18.6°C and a minimum of 1.8°C. The skies were mostly sunny and the crevice was completely exposed to sunlight. The camphor concentrate was applied to the crevice and beetle activity was observed between 1545 and 1615 hours. During this 0.50-h period, 100% of the approaching beetles were repelled from entering the crevice (Table 2). The behavior of repelled beetles included backing away from the treated surface, flying away, or stopping and remaining motionless for up to 30 s. Other beetles attempted to walk around the treated border, presumably in search of an untreated passageway into the crevice.

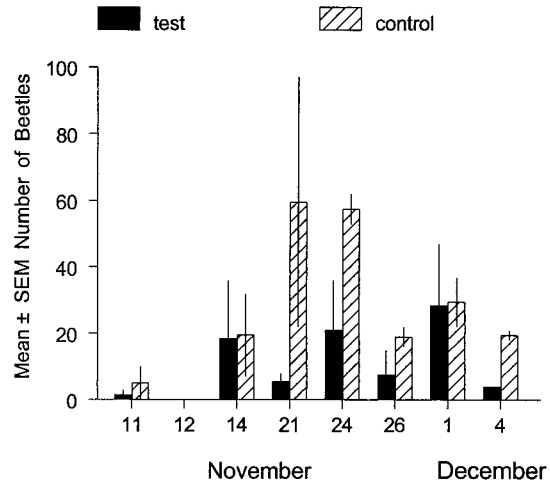


**Fig. 3.** Mean ± SEM number of beetles present within test traps versus control traps per collection date. Camphor was in the test traps and the control traps contained no chemical. For test and control traps, *n*, 16 observations.

**Behavioral Responses of Beetles in the Field—Monticello, FL.** Because of the extended summer season, beetle flight activity toward buildings was delayed until mid-November through early December 1997 in Florida. Data obtained from two field locations (pecan orchard, house) were combined to increase the replication between the treatments. On the first two collection dates, relatively few beetles were captured in either the test or control traps (Fig. 3). On subsequent dates, beetles were captured with some degree of regularity in the traps, with more beetles being captured in the control traps than in the traps baited with camphor. The overall mean ± SEM number of beetles captured in the test traps per day was 10.8 ± 3.8 beetles ($n = 16$) and the number captured in the control traps per day was 26.2 ± 6.5 beetles ($n = 16$). Significantly more beetles were captured in the control traps (*t*-test, $t = -2.1$, df = 30, $P = 0.046$).

## Discussion

Because camphor (a bicyclic ketone) elicited the greatest avoidance response in adult beetles, we surmised that camphor vapors were more of an irritant to the chemosensory organs of *H. axyridis* as compared with the other chemicals tested. Olfactory receptors for detecting vapors are probably most prevalent on the antennae and palpi in this species, because physical contact with the treated paper in the petri dish bioassays was not required for an avoidance response to occur. The results of the Y-tube tests corroborated the petri dish bioassays in that camphor had a slightly higher repellency against the beetles than did men-

thol. In contrast to the petri dish bioassays, in which the behavioral response occurred within 10 s, the Y-tube olfactometer measured a response during 10 min of exposure to contaminated air, and the bulb-tube olfactometer measured a response during 45 min of exposure to contaminated air. Beetles avoided contaminated air while in the chambers of both types of olfactometer. The observation that hexane did not elicit a behavioral response in beetles in the petri dish or olfactometer bioassays was expected. This solvent is highly volatile, readily evaporating from filter paper in seconds, without leaving any appreciable odor or residue.

Our preliminary field experiments with the use of a spray formulation of camphor or menthol (a monocyclic alcohol) showed that camphor was superior to menthol. Nevertheless, 48 h after the surface of the site (crevice) had been sprayed, beetles were no longer repelled by menthol or camphor. Camphor has been shown to function best as a fumigant or repellent in an enclosed environment (Abivardi 1977, Abivardi and Rahimian 1977, Abivardi and Benz 1984, Chauvin and Vannier 1994). These results suggest that the evaporation rate of camphor is much greater when sprayed onto a surface that is fully exposed to the elements.

Finally, we observed that camphor (in centrifuge tubes) inside traps in the field appeared to limit the entry of *H. axyridis* into these traps on some days, but not on other days (Fig. 3). Weather conditions at the field sites may have been responsible, in part, for the disparity in capture rates between collection dates. Although air temperatures were not recorded, the rate of evaporation of camphor from the uncapped centrifuge tubes was probably greatest on sunny, warm days, but least on cloudy, cool days. Similarly, beetle flight activity would be heaviest on sunny days when the air temperature was $\geq$18°C.

The development of a formulation that would sustain the activity of camphor beyond a few days is needed. One possibility is the use of potentiation agents. For example, Obeng-Ofori and Reichmuth (1999) have demonstrated that plant oils (e.g., mustard, sesame, sunflower, and coconut) potentiate the activity of camphor and other monoterpenoids in closed jars in the laboratory. Another possible means of sustaining or increasing the biological activity of a monoterpenoid is by modifying its molecular structure. Tsao et al. (1995) showed that the synthesis and subsequent use of acyl derivatives of menthol and geraniol greatly increased their acute toxicity to house fly, *Musca domestica* (L.), adults. The acute, fumigant, ovicidal, and larvicidal activities of other monoterpenoids, not considered in the current study, were also increased by derivatization.

We have been searching for chemicals that could modify the inappropriate overwintering behavior of *H. axyridis* in many locations in the United States. Adults probably orient toward their overwintering habitat by physical or visual cues. In North Carolina and Virginia, beetles were flying and landing on the southwest, west, or south facing sides of buildings, which were usually the sunnier and warmer locations in the afternoon hours (Kidd et al. 1995). In Oregon, *H. axyridis* adults were attracted to exposed, prominent, and usually light-colored buildings (La Mana and Miller 1996). In Japan, adults are attracted to whitish or light-colored objects at their natural overwintering habitats, including rock outcroppings on hilltops, in the valleys, and at the base of mountains (Obata 1986). Once at the preferred overwintering habitat, beetles may rely more on chemical cues to guide them to the precise site (crevice), leading into the overwintering cavity behind or within the structure. In Europe, adults of the twospotted lady beetle, *Adalia bipunctata* (L.), repeatedly use the same sites from one winter season to the next (Majerus 1994), which suggests that beetles are guided into the overwintering sites by odor (Majerus 1997). Possibilities for the source of such an attractant include feces from the previous winter season's population, the odor of individual beetles that die at the overwintering site each year (Hills 1969), or an endogenously produced substance (pheromone) deposited at and around the crevice leading into the overwintering cavity.

Management of this nuisance pest could be achieved by a push-pull strategy. This involves the 'pushing' of beetles away from buildings with repellent chemicals (e.g., camphor), then 'pulling' them into collecting vessels with chemical attractants or persistent pheromones. Placement of captured beetles into field cages has resulted in low survival rates during the winter (McClure 1987). Alternatively, the captives could be provided with artificial food and then stored indoors at low temperatures. Cold storage has prompted very good survival of adults without negatively impacting poststorage fecundity (Deng 1982, McClure 1987). In the spring, adults can be released into agricultural and urban landscapes and function as biological control agents.

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