Abstract

We observed the avoidance behaviour of the multicolored Asian lady beetle, Harmonia axyridis (Pallas) (Coleoptera Coccinellidae), when adults were exposed to terpenoids derived from catnip oil and grapefruit seed. In replicated laboratory bioassays, beetles avoided contact with volatiles emanating from 1 x 9 cm filter paper strips by trying to fly away, jumping back, stopping at, or turning away from the edge of strips. Although avoidance behaviours were found to vary depending on chemical concentration, turning away from the treated edge was predominant. Concentration had a highly significant effect on movement of females or both males and females when challenged with a 50 µg/µl concentration of Z,E-dihydronepetalactone or E,Z-dihydronepetalactone, respectively. Other test compounds, E,Z-iridomyrmecin and Z,E-iridomyrmecin were less effective. Finally, nootkatone and tetrahydronootkatone were least effective. A follow-up experiment using dihydronpetalactone-treated strips, which had been “aged” for 24 hr, indicated that males and females avoided 50 µg/µl of E,Z-dihydronepetalactone rather than the control and more beetles responded to volatiles by turning away. Knowledge of the behaviour of H. axyridis adults as they approach treated surfaces may provide clues to pushing them away from man-made structures and pulling them into traps with attractants or pheromones in the fall season.

Key words: dihydronpetalactone, nuisance pest, repellents, push-pull strategy, Coccinellidae, harlequin ladybird, multicolored Asian lady beetle.

Introduction

Harmonia axyridis (Pallas) (Coleoptera Coccinellidae) is an entomophagous ladybird beetle originating in Asia. In spring and summer, H. axyridis larvae and adults are important predators of aphids and other soft-bodied insects on plants in forested and agricultural landscapes (Brown, 2004; Butin et al., 2004; Mignault et al., 2006). In early to late fall, depending on geographic locality, adults migrate from feeding 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). In North America, adults also form winter aggregations in sheltered places, including buildings (Nalepa et al., 1996; Schaefer, 2004).

The propensity of H. axyridis adults to enter houses in the fall season has resulted in this insect becoming a nuisance pest (Koch and Galvan, 2008). Beetles that successfully enter houses can aggregate by the thousands in secluded dark places. 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. Reflected hemolymph has an unpleasant odor and can stain walls, furniture and draperies. In addition, human allergic reactions to H. axyridis particulate inhalants, perhaps from dead beetles, have been reported (Ray and Pence, 2004; Nakazawa et al., 2007).

Adults can aggregate in grape clusters in commercial vineyards near the time of harvest and feed on juices exuding from damaged grapes (Koch et al., 2004; Galvan et al., 2006a). If undetected, beetles can be crushed along with grapes during wine processing. Beetle-tainted wine has a discernible off-taste (Pickering et al., 2004).

There is a need to discover effective methods to manage populations of H. axyridis (Kenis et al., 2008). 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; Pree et al., 2004; Galvan et al., 2006b). Riddick et al. (2004) examined the potential of DEET (N,N-diethyl-3-methylbenzamide) to repel H. axyridis adults from house exteriors; filter paper strips treated with DEET were effective against this insect in laboratory trials. Although DEET has residual activity of two weeks in the laboratory when combined with paraffin (Riddick et al., 2004), its usefulness in the field is limited to unpainted surfaces because it is a plasticizer and dissolves paint.

Riddick et al. (2000) examined the ability 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 natural products tested in the laboratory and camphor repelled beetles from treated surfaces in the field. Neither compound has been tested at a commercial scale. To increase the arsenal of plant-derived molecules that can be used to repel H. axyridis adults, we investigated several terpenoids derived from compounds present in catnip oil and grapefruit seed, including dihydronpetalactone, iridomyrmecin, nootkatone and tetrahydronootkatone. These compounds were selected be-
cause of their known potential as repellents of other insects.

Nepetalactone is a constituent in the essential oil of catnip, Nepeta cataria L. (Lamiales Lamiaceae) and it has been shown to repel some species of cockroaches, flies, termites, and mosquitoes (Peterson et al., 2002; Peterson and Ems-Wilson, 2003; Schultz et al., 2004; Bernier et al., 2005; Chauhan et al., 2006). Dihydrolepeta lactone, which can be synthesized from nepetalactone, is also found in the abdominal defensive gland of a staphylinid beetle and is known to repel ants (Jefson et al., 1983). Iridomyrmecin is also found in catnip oil. It can function as an ant alarm or trail pheromone at high or low concentrations, respectively (Simon and Hefetz, 1991). Interestingly, iridomyrmecin and dihydrolepeta lactone have been found in the mesothoracic gland secretions of antacid beetles and can function as repellents against some ants (Hemp and Dettner, 1998). Nootkatone has been isolated from the peel oil of grapefruit, Citrus paradisi Macfad. (Sapindales Rutaceae) and other citrus, the oil of vetiver grass, and essential oil of Alaska yellow cedar and it is known to repel termites (Zhu et al., 2001; Nix et al., 2003; Ibrahim et al., 2004) and ticks (Dietrich et al., 2006). Tetrahydronootkatone, which can be synthesized from nootkatone, has repellent activity against termites (Nix et al., 2003; Zhu et al., 2003). The main goal of this study was to examine the avoidance behaviour of H. axyridis adults as they approached volatiles of dihydrolepeta lactone, iridomyrmecin, nootkatone, and tetrahydronootkatone in laboratory bioassays. Knowledge of the behaviour of H. axyridis adults as they approach treated surfaces may provide clues to pushing them away from man-made structures with repellents and pulling them into traps with attractants or pheromones.

Materials and methods

Compounds

The essential oil of catnip was purchased from Health and Herbs (Philomath, OR) and nepetalactone was isolated from it in the laboratory. The structures of nepetalactone derivatives were confirmed by gas chromatography - mass spectroscopy and nuclear magnetic resonance spectral analysis. High performance liquid chromatography confirmed that the pure oil contained a 4:6 ratio of Z,E- to E.Z- nepetalactone. E,Z-dihydrolepeta lactone, E,Z-dihydrolepeta lactone, E,Z-iridomyrmecin and Z,E-iridomyrmecin were synthesized in the laboratory from E.Z and Z,E-nepetalactone, respectively following published methods and synthetic modifications (Chauhan et al., 2004). All synthetic compounds were purified by flash column chromatography and chemical purities were confirmed (≥ 98%) by gas chromatography.

Nootkatone, which has been isolated from grapefruit seed, was purchased from Lancaster Chemicals (Belham, NH). Tetrahydronootkatone was prepared from nootkatone by catalytic hydrogenation (Chauhan et al., 2006). For clarity, compounds are represented herein using the following designations:

1ZE-DH (Z,E-dihydrolepeta lactone), 2EZ-IRI (E,Z-iridomyrmecin), 3EZ-DH (E,Z-dihydrolepeta lactone), 4ZE-IRI (Z,E-iridomyrmecin), 5NOOTKA (nootkatone), and 6THNOOT (tetrahydronootkatone). The molecular structures are displayed in figure 1.

Insect cultures

Several shipments of 300 to 400 H. axyridis adults, including males and females, were received from a commercial distributor (Rincon-Vitova Insectaries, Ventura, CA) in April and July 2004. Although the age of the beetles was not known, based on general appearance, it was assumed that all beetles were of the same generation and did not harbour any parasites. Adults were placed at random into polypropylene cages (30 x 30 x 30 cm, 24 mesh size, Bug Dorm 1 ™, MegaView Science Education Services Co., Ltd., Taiching, Taiwan), provisioned with food such as pure honey, apple slices, lepidopteran eggs and sterile water on cotton wads at the base of each cage. Cages containing mixed sexes were held inside a plant growth chamber (at 10 °C, 60-68% RH, 12 L: 12 D cycle). Just minutes before experimentation, sample beetles were randomly removed from cages, sexed, and held in groups of 10 in 50-ml polyethylene vials, inside a bench-top chilling incubator (A. Daigger & Company, Vernon Hills, IL) at 15 °C and complete darkness.

Behavioural response to compounds several minutes after application

Laboratory bioassays were conducted to determine the avoidance behaviour of individual beetles in response to compounds. Bioassays were conducted on 6 July
(1ZE-DH and 2EZ-IRI), 7 July (3EZ-DH and 4ZE-IRI), and 8 July 2004 (5NOOTKA and 6THNOOT) for trial 1; and 13 July (6THNOOT, 5NOOTKA, and 4ZE-IRI) and 14 July 2004 (3EZ-DH, 2EZ-IRI, and 1ZE-DH) for trial 2. We compared the behavioural responses of beetles to each compound, separately, at four concentrations (i.e., 0, 1, 10, 50 µg/µl). Hexane was the solvent in all cases. All concentrations of the test solutions were prepared on the same day they were used. Filter paper strips (1.0 x 9.0 cm, W x L; Whatman® grade 1) were treated with 128 µl of test solutions and with the hexane blank, control, so that all surfaces of the paper were lightly wetted. After allowing approximately 1 min for the solution to dry on the surface of the paper, each strip was positioned and taped underneath to the cut edge of a ½ circle of filter paper of the same size and grade and placed inside a clean, glass Petri dish, 20 mm deep, 100 mm diam. Each Petri dish was placed horizontally in a chemical fume hood with overhead, bright fluorescent lights. For ease of handling, and to ensure that the sexes were kept separate, each male and female beetle was tested sequentially (all males then all females), but on the same dates for a given compound. A random sample of adult beetles was subjected to treated strips. Replicates of 8 to 10 male or female beetles were tested per concentration for each compound. Each test beetle was placed in the Petri dish, on the untreated ½ circle of paper, and oriented in the direction of the treated strip using featherweight forceps (BioQuip, Rancho Dominguez, CA). The response of each beetle as it approached the treated strip was observed within a 1 min time frame. Each beetle was tested only once. Each treated strip was replaced with a new strip after every 8-10 replicate beetles, and a separate, clean Petri dish was used for each series. All experiments were carried-out at ambient conditions (25.7 ± 0.2 °C; 45.7 ± 0.5% RH).

The percentage of beetles avoiding treated strips was determined per compound per concentration. Avoidance was categorized into 4 behaviours; attempting to fly away, jumping back, stopping, or turning away from the edge of the test strip. The percentage of beetles displaying these four behaviours was determined at each concentration. Thirty-two observations of beetle movement were made per test compound.

**Behavioural response to compounds 24 hr after application**

Two compounds, 1ZE-DH and 3EZ-DH, were considered for further study because they elicited highly significant avoidance responses in females and/or males. Filter paper strips were treated with test solutions formulated from the two compounds. A series of strips were prepared at each concentration, placed inside glass Petri dishes with lids, and allowed to “age” for 24 hr. A random sample of adult beetles was subjected to strips at four concentrations using the same design and under similar ambient conditions as in the previous experiment. The total number of beetles subjected to each compound, irrespective of concentration, was 40 males and 40 females per trial. Male and female beetles were tested on the same dates for a given compound. Two identical trials were conducted for each compound on 20 July for 1ZE-DH and 27 July 2004 for 3EZ-DH. The percentage of beetles avoiding the treated strip was determined per compound per concentration. Avoidance was categorized and beetle movement observed as previously described for non-aged treatments. The percentage of beetles displaying these four behaviours was determined at each concentration. Thirty-two observations of beetle movement were made per test compound.

**Statistical analysis**

All data were analyzed following a randomized complete block design. The analysis of variance (ANOVA) with the General Linear Model (GLM) was used to test for significant differences between chemical concentration and avoidance behaviours at the cut edge of the test strip. Trial 1 and 2 was used as a source of replication for percentage data. Data generated from bioassays with each compound were analyzed separately. Data from males and females were analyzed separately. Percentage data were arcsine-transformed prior to analysis. Means were considered significantly different when $p \leq 0.05$. Tukey’s HSD procedure was used for separation of means when significant differences were detected with the ANOVA. Statistical analyses were performed with SYSTAT (2004) software. Only non-transformed means are presented.

**Results**

**Behavioural response to compounds several minutes after application**

*Z,E*-dihydronepetalactone (1ZE-DH) had a highly significant concentration effect on the movement of *H. axyridis* females (table 1). More females avoided strips treated with 50 rather than 0 µg/µl. A significant concentration effect on male behaviour was not found, maybe due to high variance (figure 2). The behaviour of both males and females at the test strip differed significantly amongst the four avoidance behaviours (attempting flight, jumping back, stopping, and turning away from the edge of the test strip. More males turned away than stopped or jumped back; also, more males attempted flight than stopped at the edge (figure 2). More females turned away than stopped or jumped back from the edge of the test strip. The total percentage of beetles avoiding strips treated with 50 µg/µl of 1ZE-DH was 95.0 ± 5.0% for both males and females.

*E,Z*-iridonyrmecin (2EZ-IRI) had a significant concentration effect on beetle movement (table 1). More males avoided strips treated with 50 and 10 rather than 0 µg/µl. Neither male nor female movement at the treated edge differed significantly amongst avoidance behaviours (figure 2). There was a marginally significant interaction of concentration and behaviour on female movement; more females turned away from strips treated with 50 rather than 0 µg/µl for all four avoidance behaviours and 1 µg/µl for stopping at the strip edge. Irrespective of behaviour, 2EZ-IRI had a highly significant concentration effect on female movement; more females avoided strips treated with 50 and 10 rather than 0 µg/µl. The total percentage of beetles avoiding
Figure 2. Mean ± SEM percentage of males and females attempting to fly away, jump back, stop, or turn away from filter paper strips treated with different concentrations of 1ZE-DH, 2EZ-IRI, 3EZ-DH, 4ZE-IRI, 5NOOTKA, and 6THNOOT (which had been “aged” for several minutes). Sample size, n = 32 observations of percentage data per compound. Male (m) and female (f) data are displayed in the left and right-hand columns, respectively.
strips treated with 50 µg/µl of 2EZ-DH was 78.9 ± 1.1 and 72.0 ± 5.5% for males and females, respectively.

*E.*Z-dihydropetala lactone (3EZ-DH) had a highly significant concentration effect on the movement of both males and females (table 1). More males avoided strips treated with 50 and 10 than 0 µg/µl; more females avoided strips treated with 50, 10 and 1 than 0 µg/µl. More males and females turned away rather than stopped, jumped back, or attempted to fly away from the edge of the test strip (figure 2). The total percentage
Table 1. Analysis of variance statistics of differences between chemical concentration and *H. axyridis* avoidance behaviours at filter paper strips treated several minutes before testing.

<table>
<thead>
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<td><em>df</em></td>
<td><em>p</em></td>
<td><em>F</em></td>
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<td>0.51</td>
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<td></td>
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<td>3</td>
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<td>4.16</td>
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<tr>
<td>Behaviour</td>
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<td>0.195</td>
<td>3.43</td>
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<td>Concentration x Behaviour</td>
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<tr>
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<td>5NOOTKA</td>
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<td>Concentration</td>
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<td>0.22</td>
<td>4.02</td>
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<td>Behaviour</td>
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Residual (i.e., error) *df* = 15 for each analysis. Means deemed significantly different when *p* ≤ 0.05. Sample size, *n* = 32 observations. Refer to figure 2 for corresponding data.

...of beetles avoiding strips treated with 50 µg/µl of 3EZ-DH was 82.5 ± 7.5 and 88.75 ± 1.25% for males and females, respectively.

*Z,E*-irdomyrmecin (4ZE-IRI) did not have a significant concentration effect on the movement of males (table 1). There was a marginally significant interaction of concentration and behaviour on female movement; but means were not distinguishable probably due to high variance. Irrespective of behaviour, concentration had a marginally significant effect on female movement. However, means were not distinguishable probably due to high variance. Irrespective of concentration, there was a marginally significant difference amongst avoidance behaviours; more females turned away than jumped back from the treated edge (figure 2). No significant differences were detected for male movement. The total percentage of beetles avoiding strips treated with 50 µg/µl of 4ZE-IRI was 89.4 ± 0.5 and 70.0 ± 20.0% for males and females, respectively.

Nootkatone (5NOOTKA) had a significant concentration effect on the movement of females, but not males. More females avoided strips treated with 50 rather than 1 µg/µl (table 1). Significant differences in male behaviour were detected; more males turned away rather than stopped or jumped back from the treated edge (figure 2). The total percentage of beetles avoiding strips treated with 50 µg/µl of 5NOOTKA was 55.0 ± 5.0 and 55.5 ± 5.0% for males and females, respectively.

Tetrahydronootkatone (6THNOOT) did not have a significant concentration effect on the movement of males (table 1). Concentration did have a marginally significant effect on females; more females avoided strips treated with 10 rather than 0 µg/µl. Marginally significant differences in behaviours were detected for males and females (table 1). Females more often turned away rather than stopped at the treated edge (figure 2). Note that means for male behaviours were not distinguishable. The total percentage of beetles avoiding strips treated with 50 µg/µl of 6THNOOT was 55.5 ± 5.0 and 45.0 ± 5.0% for males and females, respectively.

**Behavioural response to compounds 24 hr after application**

*Z,E*-dihydronepetalactone (1ZE-DH) on test strips and “aged” for 24 hr had a marginally significant concentration effect on male movement (table 2); more males...
Table 2. Analysis of variance statistics of differences between chemical concentration and *H. axyridis* avoidance behaviours at filter paper strips treated 24 hr before testing.

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Residual (i.e., error) *df* = 15 for each analysis. Means deemed significantly different when *p* ≤ 0.05. Sample size, n = 32 observations. Refer to figure 3 for corresponding data.

Avoided strips treated with 10 rather than 0 µg/µl. Neither the 50 nor the 1 µg/µl concentration elicited a significant avoidance response. Males more often turned away rather than stopped, jumped back, or attempted to fly away from the edge of the treated strip (figure 3). Females were not significantly affected by concentration and significant differences amongst behaviours were not evident. The total percentage of beetles avoiding strips treated with 50 µg/µl of 1ZE-DH was 40.0 ± 0.0 and 45.0 ± 15.0 for males and females, respectively.

**Figure 3** Mean ± SEM percentage of males and females attempting to fly away, jump back, stop at, or turn away from filter paper strips treated with different concentrations of 1ZE-DH and 3EZ-DH (which had been “aged” for 24 hr). Sample size, n = 32 observations of percentage data per compound. Male (m) and female (f) data are displayed in the left and right-hand columns, respectively.
E,Z-dihydronepetalactone (3EZ-DH) on test strips and “aged” for 24 hr, provoked a highly significant interaction of concentration and behaviour on male beetles (table 2). More males turned away from strips treated with 50 rather than 10, 1, or 0 µg/µl (figure 3). Irrespective of behaviour, more males avoided strips treated with 50 rather than 1 or 0 µg/µl. Also, more males avoided strips treated with 10 rather than 1 or 0 µg/µl. Irrespective of concentration, turning away was predominant to stopping, jumping back, or flying away from the treated strip. There was also a highly significant interaction of concentration and behaviour on female beetles (table 2); more females turned away from strips treated with 50 rather than 10, 1, or 0 µg/µl. To a lesser extent, females flew away from strips treated with 50 rather than 1 or 0 µg/µl (figure 3). Irrespective of behaviour, more females avoided strips treated with 50 rather than 10, 1, or 0 µg/µl. Irrespective of concentration, more females turned away rather than stopped, jumped back or attempted to fly away from the treated strip. The total percentage of beetles avoiding strips treated with 50 µg/µl of 3EZ-DH was 55.0 ± 5.0 and 35.0 ± 5.0% for males and females, respectively.

Discussion

The observation of highly significant concentration and behavioural effects on female or both male and female movement for 1ZE-DH and 3EZ-DH in several minutes after application, coupled with greater than 80% of both sexes avoiding the edge of paper strips treated with 50 µg/µl of either of these compounds, suggests that dihydronepetalactones have short term repellent activity against H. axyridis in the laboratory. An interesting and unexpected finding of this study was that males and females both predominantly exhibited a turning away, rather than any of the other avoidance responses, to strips treated with the highest concentration of dihydronepetalactone. The predominance of this response was also observed as H. axyridis adults approached strips treated with DEET (Riddick and Brown, unpublished data). Perhaps turning away from a treated strip in a laboratory bioassay is an estimate of the behavioural response of beetles when approaching treated cracks and crevices on a house exterior in the field. A crevice with a 4- to 5-mm gap size was found to be large enough to permit entry of beetles into artificial shelters in the laboratory (Nalepa, 2007). A strategy that involves pushing H. axyridis adults away from cracks and crevices with repellents and pulling them into traps with attractants or pheromones might be a viable option for managing this nuisance pest. Turning away rather than flying away from a repellent suggests that a trap for capturing beetles must be stationed in the vicinity and easily accessible by crawling. Mounting traps on the house exterior could provide access to crawling beetles as well as others that have recently landed on the house exterior. Captives could then be cold-stored over the winter and later released in the spring season for biocontrol of aphids and other soft-bodied pests.

This study adds to the arsenal of repellents that have promise for pushing beetles away from houses. Recent studies indicated that β-caryophyllene (a sesquiterpene) and methoxypyrazines are airborne volatiles emitted by H. axyridis (Brown et al., 2006; Cai et al., 2007; Verheggen et al., 2007) and β-caryophyllene may function as an attractant or as a component of an aggregation pheromone (Verheggen et al., 2007) and could be used to pull beetles into traps.

The observation that E,Z-dihydronepetalactone, which had been “aged” for 24 hr, provoked highly significant concentration, behaviour, and interaction effects on beetle movement, suggests that this isomer has some residual repellent activity under laboratory conditions. Because the percentage of males and females avoiding “aged” test strips at the highest concentration was only 55 and 35%, respectively, the likelihood that E,Z-dihydronepetalactone will repel H. axyridis adults for 24 hr under field conditions is doubtful. Development of a formulation with residual activity of 1 to 2 weeks for use on man-made structures is needed. Prolonging the activity of dihydronepetalactone, or any other plant-derived volatiles, is possible. For example, Hori (2005) indicated that hinokitiol (β-thujaplicine) had no significant residual activity against an anobid beetle, Lasio derma serricorne (F.), 2 days after application, but residual activity lasted for up to 92 days when hinokitiol was sealed between a polyethylene film bilayer.

Previous research has shown that a steam-distillate of catnip oil was as good a repellent as DEET or citronella against house flies, Musca domestica L. and American cockroaches, Periplaneta americana L. (Schultz et al., 2004). Nepetalactones are primarily responsible for behaviour modification; the E,Z-nepetalactone isomer was more repellent to cockroaches than the dominant isomer, Z,E-nepetalactone and the essential oil of catnip (Peterson et al., 2002; Peterson and Ems-Wilson, 2003). The trans, trans (i.e., E,E) isomer of dihydronepetalactone, isolated from the defensive glands of a staphylinid, Creophilus maxillosus (L.), repelled ants [Monomorium destructor (Jerdon)] in laboratory bioassays (Jefson et al., 1983).

In conclusion, derivatives of terpenoids isolated from catnip oil (especially E,Z-dihydronepetalactone) displayed an ability to modify the behaviour of H. axyridis males and females in the laboratory. Although testing under field conditions will be necessary, this study suggests that a sustainable formulation of dihydronepetalactone may be useful in pushing beetles away from treated surfaces on house exteriors in autumn.

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References


SIMON T., HEFETZ A., 1991.- Trail-following responses of 
*Tapinoma simrothi* (Formicidae: Dolichoderinae) to pygidial 

SYSTAT, 2004.- *Statistics, Version 11.*- SYSTAT Software 
Inc., Richmond, CA, USA.

VERHEGGEN F. J., FAGEL Q., HEUSKIN S., LOGNAY G., FRANCIS 
F., HAUBRUGE E., 2007.- Electrophysiological and behavioral 
responses of the multicolored Asian lady beetle, *Har 
monia axyridis* Pallas, to sesquiterpene semiochemicals.- 

WILLIAMS R. N., FICKLE D. S., 2002.- Laboratory evaluations 
for repellency and toxicity to the multicolored Asian lady 

WILLIAMS R. N., ELLIS M. A., FICKLE D. S., 2002a.- Bioassay 
evaluations for toxicity to multicolored Asian lady beetle, a 
novel pest of grapes, 2001.- *Arthropod Management Tests*, 
27: L14.

WILLIAMS R. N., FICKLE D. S., KOVACH J., 2002b.- Bioassay 
evaluations for toxicity to multicolored Asian lady beetle, a 
nuisance pest, 2001.- *Arthropod Management Tests*, 27: 
L19.

ZHU B. C. R., HENDERSON G., CHEN-FENG, MAISTRELLO L., 
LAINE R. A., 2001.- Nootkatone is a repellent for Formosan 
subterranean termite (*Coptotermes formosanus*).- *Journal 

ZHU B. C. R., HENDERSON G., SAUER A. M., YU Y., CROWE W., 
rivatives and their repellence to the Formosan subterranean 

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