The consequences of sublethal exposure to insecticide on the survivorship and mobility of *Halyomorpha halys* (Hemiptera: Pentatomidae)

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**Abstract**

**BACKGROUND:** The direct lethal effects of conventional and organic insecticides have been investigated thoroughly for all life stages of *Halyomorpha halys*. However, the sublethal effects of insecticides on the behavior of *H. halys* have not been well documented. Our aims were to evaluate the impact of a brief 5 min exposure to residues of bifenthrin, dinotefuran, methomyl, thiamethoxam and thiamethoxam + λ-cyhalothrin on survivorship, horizontal and vertical movement, and flight capacity of adult *H. halys* under laboratory conditions.

**RESULTS:** Over half of the insecticide-exposed adults were classified as affected, moribund or dead after the 5 min exposure, compared with only 6% of the adults in the water-only control. We found that the horizontal movement, vertical climbing and flight capacity of adults exposed to insecticides were decreased by 20–60% overall relative to the water-only control. The most lethal insecticide was bifenthrin.

**CONCLUSION:** Many insecticide-exposed *H. halys* adults retained significant mobility and flight capacity, with flight most pronounced immediately after exposure. These results suggest that brief exposure periods to efficacious insecticides will result in high dispersal and low mortality. Therefore, management strategies that enhance the retention of *H. halys* on insecticide-coated surfaces should be considered to ensure that adults are exposed to a lethal dose of insecticide.

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**Keywords:** attract-and-kill; toxicology; behavior; brown marmorated stink bug; integrated pest management; behavior

1 **INTRODUCTION**

The accidental introduction of *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) has created agricultural and nuisance issues in the United States, Canada and several European countries. 1–6 *H. halys* populations in the Eastern United States originated near Beijing, China, 7 and were first detected in Allentown, Pennsylvania, in the mid-1990s. 8 Currently, *H. halys* can be found in 42 US states, and has caused severe agricultural damage in nine of those states (www.stopbmsb.org, last accessed 30 March 2016). The pest feeds on over 100 host species, 9–11 many of economic importance. As a consequence, there has been a concerted effort to find effective management tactics.

In part, this has included determining optimal insecticides in both conventional11–21 and organic production systems, 22–24 as well as for homeowners and gardeners. 25 These studies have involved examining the effects of exposure to insecticides labeled for various cropping systems using a variety of laboratory and field-based methods on the mortality, mobility and behavior of *H. halys* and efficacy against various life stages (eggs, nymphs, adults). Some of the most effective insecticides for *H. halys* include bifenthrin, methomyl, permethrin, dinotefuran, thiamethoxam and λ-cyhalothrin.

Most of the results generated to date are based on longer exposure times (e.g. 1.5–4.5 h or longer) to targeted materials under restricted conditions. While useful at establishing baseline mortality levels, this approach does not take into account the ability of *H. halys* to leave areas treated with insecticides, especially considering its strong dispersal capacity during the adult26,27 and nymphal28 life stages, nor the fact that this insect often invades from untreated, border habitats.29–32 The ability to move to and from protected crops can result in failure to take up a lethal dose of insecticide. While exposure may result in effects that may temporarily impair behavior and mobility, it may not kill the individual outright. Some authors have suggested that, in order properly to understand the effect of an insecticide, sublethal effects must be taken into account. 33 Moreover, studying behavior may be a particularly important indicator of toxicity as it has been described as 10–1000 times more sensitive than LC50 estimates. 34–36 This is especially salient when considering the intensive management required effectively to suppress *H. halys* in economically important crops. Prior studies have demonstrated that there is some amount of recovery in terms of *H. halys* condition17,22 and dispersal capacity after exposure to certain insecticides (e.g. Lee et al.16), even after exposure to lethal doses over extended periods. This increases the probability that an adult experiencing sublethal
insecticide exposure may quickly recover and continue to pose a risk to agricultural systems, but the magnitude and extent of this risk are unknown. However, in certain behaviorally-based management strategies such as attract-and-kill, where *H. halys* are attracted to an area with a killing agent via semiochemicals, the pest may repeatedly contact insecticide-coated surfaces, thereby ultimately taking up a lethal dose of insecticide even if individual exposure intervals are short.

Understanding the sublethal effects of insecticides on the behavior of *H. halys* is also applicable for detection–exclusion programs aimed at mitigating the risk of accidentally importing *H. halys* on cargo. For example, New Zealand and Australia are highly interested in behaviorally-based strategies such as attract-and-kill technology to prevent the establishment of breeding pest populations that may endanger the quality of their agricultural exports. There is a concerted effort by the agencies in these countries to monitor for this pest, and exclude it, and if insecticides are applied for *H. halys* that result in complete mortality or sublethal effects, the prevention program for these countries could fail. Thus, having a complete analysis of effective chemistries for *H. halys* is vital, especially evaluating the immediate consequences after a limited exposure period to insecticide. In addition, a complete analysis of the sublethal effects should also be of interest in ongoing *H. halys* management efforts in Europe. In the European Union, there has been great concern about the use of insecticide, and a push for adoption of integrated pest management programs. As a result, the sublethal implications on the behavior of *H. halys* for tactics such as attract-and-kill, which can significantly reduce the amount of insecticide applied in orchards, may be of great interest.

While prior research has established the effect of long exposure times (>1.5 h) on the behavior of *H. halys*, there is nothing known about how brief exposures (~5 min) to insecticide may immediately alter the behavioral dynamics of this invasive species. Literature with other species has widely documented a dose-dependent increase in mortality with increasing doses of insecticide. Because behavior is a more sensitive indicator of toxicity than mortality, one may expect an even more pronounced change in the behavior of *H. halys* between prolonged and limited exposure to insecticide.

In the present study, our aims were to evaluate the impact of limited exposure to five efficacious insecticides from different chemical classes on (1) survivorship, (2) horizontal movement, (3) vertical climbing capacity and (4) flight capacity of *H. halys* in the laboratory immediately post-exposure. To achieve these aims, we subjected *H. halys* adults to insecticide residues for limited exposure periods (5-min duration) to assess the sublethal impacts of the insecticides on mortality and mobility. We included bifenthrin, dinotefuran, methomyl, thiamethoxam and the combination thiamethoxam + *α*-cyhalothrin, as these have been shown to have the greatest efficacy in prior studies based on lethality index and longevity across the range of chemical classes. Each of these compounds was among the top five most effective compounds in their respective chemical classes.

## 2 MATERIALS AND METHODS

### 2.1 Experimental insects and pretrial handling

Wild *H. halys* adults were collected from selected sites by deploying artificial overwintering structures at anthropogenic sites (e.g. sheds, buildings, storage areas) with known overwintering populations. Shelters were deployed at Pleasant Valley Elementary School in Knoxville, Maryland (39° 21’ 31.76” N, 77° 44’ 33.08” W), at a residence on a promontory surrounded by a commercial orchard with known *H. halys* pressure in West Virginia (39° 23’ 30.92” N, 78° 5’ 9.03” W) and at another residence in Knoxville, Maryland (39° 22’ 28.42” N, 77° 40’ 53.59” W) (e.g. the site of >11 000 overwintering *H. halys* in Inkley, 2012). The shelters (Bergh JC et al., unpublished) were similar in appearance to a birdhouse (22 cm x 24 cm x 19 cm, *H x W x L*), and constructed of 0.635 cm thick plywood. A shallow-sloped roof that overhung the removable front panel had a 0.635 cm gap between it and the top of the front panel, enabling adults to enter or leave the shelter via this opening or a bottom slit. Sixteen cardboard inserts (0.318 cm thick, 17.78 cm long, 21.59 cm wide) with cardboard spacers (0.318 cm thick, 1.27 cm wide, 17.78 cm long) glued to the surface along both edges of one side were stacked vertically from back to front inside each shelter, filling most of the interior. Shelters were elevated on top of two apple bushel crates, and were placed at overwintering sites from 19 September 2014 to 28 November 2014, where adult *H. halys* were allowed naturally to settle during their fall dispersal period.

Shelters were retrieved and stored in an unheated outbuilding from November to February (mean ± SE: 1.83 ± 0.1 °C). Adults were retrieved from shelters in groups of 100 with a 1:1 sex ratio, placed in screen cages (30 x 30 x 30 cm) and held at 16:8 (L:D), 25 ± 1 °C and 70 ± 10% RH. Adults were provided with organic carrots, sundried tomatoes, dried sunflower seeds and water for a 2 week acclimation period to induce foraging behavior prior to testing. Only those adults that were visibly active and healthy (all limbs and antennae intact) were chosen for testing in insecticide bioassays.

### 2.2 Insecticide bioassay

In this study, guidelines published by the International Organization of Biological Control and described in detail by Leskey et al. were followed. Based on label recommendations for tree fruit in the mid-Atlantic, insecticides were mixed with water at a concentration equal to the use of 936 L of finished spray material per hectare (Table 1) and atomized onto sterile polystyrene petri dishes and lids (100 x 15 mm) at a volume equal to a delivery rate per unit area of ≈ 360 μL per Petri dish arena or the equivalent of 0.113 g of fluid. Sprayed arenas were allowed to dry overnight (~16 h) in a fume hood before testing.

Six different treatments were evaluated, including a distilled water control. The five insecticides were: bifenthrin, dinotefuran, methomyl, thiamethoxam (thx) and the combination thiamethoxam + *α*-cyhalothrin (thx + *α*-cy) (Table 1). These chemicals were chosen on the basis of their toxicity to *H. halys* after a 4.5 h exposure. In each trial, adults were exposed to commercially

<table>
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<tr>
<th>Table 1. Recommended and tested rates of insecticides used in this study to induce sublethal effects after a 5 min exposure in <em>H. halys</em></th>
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<tr>
<td>Trade name</td>
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<tr>
<td>Brigade</td>
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<td>Venom</td>
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<td>Lannate</td>
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<td>Endigo</td>
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<td>Actara</td>
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<sup>a</sup> Rate in g of active ingredient in 936 L per ha.
<sup>b</sup> Rate in mL of active ingredient in 936 L per ha.
labeled rates of insecticide in treated petri dishes for 5 min. This exposure time was chosen on the basis of preliminary data indicating ~30% mortality at this exposure interval, allowing a sufficient number of individuals to survive to assess the sublethal impact of the insecticides on the behavior of H. halys (Morrison WR et al., unpublished data). A total of 20–22 trials were performed for each treatment, each containing 5–6 adult H. halys, depending on the assay performed; 10–11 trials of each insecticide were for adults tested in the flight assay, and the other 10–11 trials were for those in the horizontal/vertical movement assay (see sections below). In total, 683 adults were evaluated. Adults were placed individually into petri dishes with lids to prevent escape. Immediately after exposure, and also 6 days after exposure, the physical condition of H. halys adults was rated, on the basis of overall effect of insecticide exposure, as alive, affected, moribund, or dead. These categories were defined as described in Leskey et al.17 and are given as follows: (1) alive: no signs of impairment, could move horizontally and vertically and appeared to feed normally; (2) affected: capable of moving, but with irregular, lethargic movements; (3) moribund: nearly immobilized, unable to right itself if flipped, only capable of slight movements of legs or antennae; (4) dead: no movement, even in response to probing.

After the assays described below (e.g. flight mills, horizontal/vertical movement trials), adults were provided with food and water provided ad libitum, and their condition was assessed again 6 days later. On a daily basis, the cups were monitored for mold growth, with food and housing replaced as needed. In order to compare the lethality of limited exposures of insecticides to adults exposed for 4.5 h in a prior study, we calculated a lethality index. The correlation of the lethality index and the time since exposure (0 or 6 days) and their interaction as categorical, explanatory variables, with the date of trial used as a random blocking variable. The residuals were inspected to ensure that parametric assumptions associated were fulfilled. Because assumptions were fulfilled, untransformed data were used. Upon a significant result from the ANOVA, Tukey’s HSD was used for pairwise comparisons. For this, and all subsequent statistical tests, α = 0.05.

2.3 Horizontal movement
After exposure to insecticide, half of the adults (N = 5 per rep) were used for testing horizontal movement by transferring them to untreated glass petri dish arenas (100 × 15 mm). Petri dishes were washed after each use with soap and water. To avoid the risk of cross-contamination, only a single insecticide was tested on a given day, and replicates were accumulated over time for each insecticide. At least one control was run once every 2 weeks, while insecticide trials were conducted for a total of 15 replicates. The horizontal movement of five adults was tracked simultaneously for 2 h immediately after insecticide exposure with a video visualization system (RE-350; Canon, Inc., Tokyo, Japan) suspended above the five arenas (petri dishes). A total of 10–11 replicates per insecticide were run for a total of 305 adults over the course of the experiment. The tests were conducted in a darkened room, with arenas backlit by fluorescent lights to ensure efficient detection and tracking of adults. The area was heated to maintain a temperature of 24 °C and humidified to >40% RH for all trials.

Using EthoVision software (v.3.1.16; Noldus Information Technology Inc., Leesburg, VA), we recorded the distance that each adult moved during the trial period. To account for ‘cursor bounce’ (variation in the position of the center of the acquired subject) in EthoVision, an input filter was applied for data acquisition. The movement distance was accumulated only if the position of the subject changed by >10% of the mean body length of H. halys (=0.14 cm) in a straight-line measure. Because the data did not conform to the assumptions of a normal distribution, these values were log transformed. The response variable was analyzed with a linear model (JMP Genomics 5.0; SAS Institute Inc.). The model contained the treatment (water only control, bifenthrin, dinotefuran, methomyl, thiamethoxam and thx + λ-cy), sex (male or female) and their interaction as explanatory variables, using the date the experiment was run as a random blocking variable. Upon a significant result from the ANOVA, Tukey’s HSD was used for pairwise comparisons.

2.4 Vertical mobility
Immediately following each horizontal movement trial, the same five adults were transferred to be tested in the vertical mobility assay. Individuals were placed into separate clear polycarbonate cylinders (30 cm tall × 7 cm diameter) to record the climbing distance after insecticide exposure. Three 5 min trials (total of 15 min) were conducted with each individual to determine the overall distance climbed. The cylinder was inverted when an adult reached the top, and it was noted if the climbing ability of an individual was impaired because it was incapacitated. A total of 305 adult H. halys were tested. After the 15 min trial period was completed, adults were individually placed into clear 30 mL plastic cups (4 × 4 cm, H × D: Maryland Plastics Inc., Federalsburg, MD), with food and water provided ad libitum as described above. Because the data did not conform to the assumptions of a normal distribution, they were log transformed, after which assumptions of normality and homoscedasticity were met. The data were analyzed with a linear model, using the total distance climbed over the 15 min period as the response. The insecticide (control, bifenthrin, methomyl, dinotefuran, thiamethoxam or thx + λ-cy) and whether the adults were incapacitated (classified as moribund or dead) were used as explanatory variables (JMP Genomics; SAS Institute).

2.5 Flight capacity
The other cohort of exposed adults (N = 6 per rep) were used for testing of flight capacity. The nylon head of a pin (No. 1 insect pin; BioQuip Products, Rancho Dominguez, CA) was glued to the pronotum of each individual (3M Scotch-Weld Hot Melt Applicator LT 2006; St Paul, MN), ensuring not to impair proper wing functioning, and avoiding the eyes and antennae of the adults. The individual was tethered by inserting the point of the pin into the hollow tip of a hypodermic needle attached to the rotation arm of each flight mill (details given elsewhere17,28,61), with the other end containing a balancing weight. Each trial was started between 08:00 and 13:00 by gently blowing on the insect to initiate flight, and ran for 2 h in parallel with the horizontal movement assay described above after limited exposure to insecticide. There were a total of 10–11 reps per treatment, with a total of 378 individuals tested for flight capacity. At the end of a trial, insects were detached from the apparatus and placed in 30 mL cups with food, as described above. Data were streamed in real time to a computer containing the software DASYLAB (Measuring Computing, Norton, MA), which was used to record the flight parameters,
3 RESULTS

3.1 Efficacy among insecticides

The overall model explained a significant amount of variation in the percentage of adult H. halys alive after sublethal exposure to insecticides (ANOVA: $F = 21.9; df = 11, 238; P < 0.0001$). The treatment significantly altered the percentage of adults alive ($F = 32.7; df = 5, 238; P < 0.0001$) (Fig. 1). Immediately after the insecticide exposure, 60% of all adults were classified as alive among insecticides evaluated (Tukey’s HSD) (Fig. 1). Immediately after exposure, the number of adults classified as alive was lowest for thx + $\lambda$-cy and greatest for bifenthrin. However, 6 days after insecticide exposure, the number of adults classified as alive was lowest for bifenthrin and dinotefuran. In contrast, exposure to methomyl and the thiamethoxam-containing compounds resulted in the greatest percentage of adults that remained alive at the conclusion of the study (e.g., day 6). Across insecticides, 40% of all the adults were considered to be alive at the conclusion of the experiment.

Likewise, the overall model significantly explained the percentage of adults classified as affected (ANOVA: $F = 17.4; df = 11, 238; P < 0.0001$), with the treatment having a significant effect ($F = 24.3; df = 5, 238; P < 0.0001$). In total, 32% of all adults were considered to be affected immediately after exposure. Brief exposure to thiamethoxam-containing compounds (i.e., thiamethoxam and thx + $\lambda$-cy) resulted in the greatest number of affected adults directly after treatment, with 87 and 89 times more than the water-only control respectively (Tukey’s HSD) (Fig. 1). On the other hand, bifenthrin resulted in the fewest adults considered to be affected directly after treatment compared with the other compounds. By day 6, there were similar numbers of affected adults across the insecticides, with a total of 16% of adults considered to be affected.

The overall model was effective in significantly explaining the H. halys adults classified as moribund after limited exposure to insecticide (ANOVA: $F = 9.79; df = 1, 238; P < 0.0001$). Similarly to the other condition categories, the treatment significantly affected the number of adults considered to be moribund ($F = 10.9; df = 1, 238; P < 0.0001$) (Fig. 1). Immediately after exposure, 7% of all adults were considered to be moribund across the insecticides. In particular, exposure to thx + $\lambda$-cy resulted in the greatest number of adults considered to be moribund directly after treatment, whereas the other insecticides were not significantly different from the control. After 6 days, this pattern changed such that those individuals exposed to dinotefuran and bifenthrin resulted in the greatest number of adults considered to be moribund. By day 6, 16% of all adults were considered to be moribund.

Finally, the overall model also significantly explained adult H. halys that were classified as dead after a limited insecticide exposure (ANOVA: $F = 29.8; df = 11, 238; P < 0.0001$). The treatment similarly affected the number of adults that died ($F = 12.9; df = 1, 238; P < 0.0001$). No adults were found dead immediately after insecticide exposure (Fig. 1). After 6 days, bifenthrin and thx + $\lambda$-cy inflicted the greatest mortality on adults. A limited 5-min exposure resulted in 27% mortality of all tested adults by the end of the experiment, compared with only 8% mortality in the water-only control.

3.2 Efficacy within insecticide

The time since insecticide exposure (e.g., immediately after or 6 days after) also significantly affected the number of adults classified as alive ($F = 23.5; df = 1, 238; P < 0.0001$), affected ($F = 47.1; df = 1, 238; P < 0.0001$), moribund ($F = 7.69; df = 1, 238; P < 0.0006$), or dead ($F = 195.1; df = 1, 238; P < 0.0001$). Moreover, there were significant time by insecticide interactions for adults classified as alive ($F = 10.4; df = 5, 238; P < 0.0001$), affected ($F = 4.70; df = 5, 238; P < 0.0001$), moribund ($F = 9.09; df = 5, 238; P < 0.0001$), or dead ($F = 12.9; df = 1, 238; P < 0.0001$).

Based on the lethality index (LI), the most effective insecticide after a brief exposure was bifenthrin (LI: 66.1). Immediately after exposure to bifenthrin, a total of 38% of adults were either moribund, affected, or dead, whereas this figure more than doubled by the end of the experiment to 85% of the adults treated with bifenthrin. The majority of adults exposed to bifenthrin (68%) were classified as dead by the end of the experiment. Dinotefuran was the next most effective insecticide after a limited exposure based on the lethality index (LI: 40.0). Almost half of the adults treated with dinotefuran were affected or moribund directly after exposure.
Sublethal insecticide exposure affects survivorship and behavior of *H. halys* | www.soci.org

**Table 2.** The effect of a limited 5-min exposure to insecticide on the mobility and flight capacity of *H. halys* in the laboratory

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Horizontal movement$^c$(cm)</th>
<th>Distance climbed$^c$(cm)</th>
<th>Distance flown$^c$(m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control$^d$</td>
<td>2571.0 $\pm$ 515.0 a$^a$</td>
<td>270.3 $\pm$ 29.8 a $^a$</td>
<td>362.9 $\pm$ 68.2a $^a$</td>
</tr>
<tr>
<td>Thx</td>
<td>1363.9 $\pm$ 298.1 b</td>
<td>219.0 $\pm$ 33.2 ab</td>
<td>212.3 $\pm$ 61.0b</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>1297.2 $\pm$ 108.1 bc</td>
<td>23.9 $\pm$ 10.0 bc</td>
<td>414.6 $\pm$ 99.7a</td>
</tr>
<tr>
<td>Methomyl</td>
<td>908.7 $\pm$ 237.9 c</td>
<td>261.5 $\pm$ 42.7 a</td>
<td>214.4 $\pm$ 40.5b</td>
</tr>
<tr>
<td>thx + λ-cy</td>
<td>870.3 $\pm$ 110.8 c</td>
<td>176.3 $\pm$ 25.2 ab</td>
<td>173.2 $\pm$ 40.8b</td>
</tr>
<tr>
<td>Dinotefuran</td>
<td>723.6 $\pm$ 138.4 c</td>
<td>79.2 $\pm$ 20.2 b</td>
<td>369.6 $\pm$ 77.5a</td>
</tr>
</tbody>
</table>

$^a$ Distance walked in a 2 h trial using EthoVision in the laboratory.
$^b$ Distance climbed over three 5 min trials in a vertical assay.
$^c$ Distance flown on flight mills in the first 15 min after exposure to the treatment.
$^d$ Water-only control.

Similarly to bifenthrin, 85% of adults were classified as moribund, affected, or dead 6 days after exposure to dinotefuran, though with fewer actually dead and correspondingly more moribund and affected. According to the lethality index, thx + λ-cy (LI: 35.2) was only about half as effective at killing *H. halys* as the most effective compound tested. Immediately after exposure to thx + λ-cy, over three-quarters of adults were either affected or moribund, with most of those being classified as affected. This attenuates to less than half of adults classified as affected, moribund, or dead by the end of the trial period. The two chemicals with the lowest lethality indices were thiamethoxam (LI: 25.5) and methomyl (LI: 19.5). A total of 61% thiamethoxam-exposed adults were classified as affected or moribund directly after exposure, while this decreased to 47% of adults affected, moribund, or dead by the end of the trial. For methomyl-exposed adults, the percentage classified as affected, moribund, or dead was constant at about 45% both directly and 6 days after treatment.

**3.3 Horizontal movement**

Our overall model was able significantly to explain much of the variation in the walking distance of adult *H. halys* (ANOVA: $F = 14.53$; $df = 11, 293$; $P < 0.0001$). Limited exposure to insecticides significantly decreased the horizontal movement of adults, regardless of active ingredient ($F = 28.14$; $df = 5, 293$; $P < 0.0001$) (Table 2). Across insecticides, adults retained over 40% of their walking ability compared with the water-only control. Adults exposed to methomyl, thx + λ-cy, or dinotefuran resulted in the greatest decrease in horizontal movement, moving an average of only 35, 34 and 28% the distance of individuals in the control. However, adults exposed to thiamethoxam and bifenthrin retained over half of their mobility compared with the control. In addition, these sublethal effects were modulated by sex of the adult ($F = 10.76$; $df = 1, 293$; $P < 0.0012$). In particular, insecticides impaired the horizontal movement of females (mean ± SE: 902 ± 80 cm) slightly more than males (1165 ± 150 cm). This effect was the same across insecticides (sex x insecticide interaction: $F = 1.42$; $df = 5, 293$; $P = 0.217$).

**3.4 Vertical mobility**

The overall model significantly explained the distance climbed by adults after limited exposure to insecticides (ANOVA: $F = 25.10$; $df = 8, 288$; $P < 0.0001$). The active ingredient of the insecticide significantly affected the vertical movement of adults ($F = 34.68$; $df = 5, 288$; $P < 0.0001$) (Table 2). Across other factors, insecticide-treated adults still moved 55% the distance that adults moved in the control. In particular, exposure with dinotefuran and bifenthrin resulted in significant decreases in vertical climbing capacity, where adults only climbed 29 and 8%, respectively, of the distance of individuals in the control (Tukey’s HSD) (Table 2). The physical condition of the adults also significantly affected their ability to climb ($F = 29.89$; $df = 2, 288$; $P < 0.0001$). Specifically, adults that were rated as alive (mean ± SE: 270 ± 17 cm) climbed 31 and 84 times farther, respectively, than individuals who were moribund (8.7 ± 3 cm) or incapacitated (3.2 ± 3 cm).

**3.5 Flight capacity**

The flight distance of adult *H. halys* was significantly affected after sublethal exposure to insecticide (ANOVA: $F = 7.55$; $df = 5, 50$; $P < 0.0001$), though whether flight capacity decreased depended on the specific compound (Tukey’s HSD) (Table 2). Across exposure to different insecticides, adults retained almost 80% of their flight capacity compared with the control. Flight activity of *H. halys* adults was significantly influenced by the amount of time since exposure to insecticides (ANOVA: $F = 126.7$; $df = 3, 372$; $P < 0.0001$), with most flight occurring within the first 15–30 min after the trial began (Fig. 2). Specifically, adults flew 70, 84 and 95% of the distance traveled in the full 2 h trial period after 15 min, 30 min and 1 h post-insecticide exposure respectively. Adults exposed to dinotefuran flew the greatest percentage within the first 15 min at a total of 95% of the distance flown in the 2 h period, while adults exposed to the combination of thx + λ-cy flew at least 46% of the total distance in the same period (Fig. 2). After 15 min, the flight capacity of adults exposed to bifenthrin increased numerically, but, along with dinotefuran, was not significantly different from the flight distance of individuals in the control (Tukey’s HSD) (Table 2). However, limited exposures to methomyl, thiamethoxam and thx + λ-cy resulted in flights that were only 59, 58 and 47% of the distance, respectively, to individuals in the control (Tukey’s HSD) (Table 2). The interaction between time spent on the flight mill and insecticide was not significant ($F = 0.438$; $df = 15, 372$; $P = 0.709$).

**4 DISCUSSION**

Previous studies have described the effects of insecticides on the survivorship of *H. halys* in the laboratory and control of damage in the field based on longer exposure times aimed at producing lethal effects. However, this is the first study to investigate the effects of brief insecticide exposure on the behavior and mobility of this invasive pest. Prior research with other species has also enumerated a range of biological processes, including reproduction, movement, feeding and flight, that have either been stimulated or depressed (for a review, see Desneux *et al.* and Haynes).

Even the brief 5-min insecticide exposure period was effective in inducing some level of mortality, but there was a substantial amount of recovery as well. For instance, after 6 days, 27% of the adults had died, while 40% remained alive and 33% were either affected or moribund. By contrast, in the control, only 8% had died, while 89% remained alive and 3% were either moribund or affected. Previously, in a study of field residue, adult *H. halys* exposed for 24 h and checked 5 days afterwards had an average mortality of 75.7% for insecticides included in the present study.
For another study using a similar dried residue assay in laboratory petri dishes, there was an average 99% mortality rate of adult *H. halys* after exposure for 4.5 h to the same compounds included here.\(^\text{16}\) Based on previously published lethality index values,\(^\text{17}\) our tested compounds line up in the same sequence of lethality, except methomyl, which was less effective than expected. The most lethal compound among those tested in this study was bifenthrin.

On the whole, we found that exposure to insecticides reduced adult *H. halys* horizontal and vertical movement by only about 50–60% compared with the control. However, for three of the insecticides (methomyl, thiamethoxam and thx + \(\lambda\)-cy), adults retained over 65% of their climbing capacity, and for two (thiamethoxam and bifenthrin) adults retained over 50% of their walking capacity. The only other study that investigated the mobility of *H. halys* in relation to insecticides examined the direct effects on mobility for bugs exposed for 1.5–4.5 h in 10 min behavioral assays.\(^\text{16}\) That study noted a marked decrease in horizontal movement over the trial period, with *H. halys* moving an average of 2.6 cm after being treated separately with bifenthrin, dinotefuran, thiamethoxam and methomyl. Additionally, adults were so incapacitated that they were not able to climb. This is in direct contrast to the adults in the present study, which walked an average of 10.38 m and climbed 1.51 m after limited exposure to insecticide. Moreover, Lee et al.\(^\text{25}\) studied nymphal and adult *H. halys* movement separately from insecticides, and found that adults have a baseline walking capacity of 20 m in 60 min in the laboratory, as well as an ability to climb 600 cm in 15 min. Other insects have also shown reductions in mobility and walking movement after exposure to sublethal doses of insecticides, including the psocids *Liposcelis bostrychophila* Badonnel and *L. entomophila* (Enderlein).\(^\text{39}\)

Additionally, the collembolan *Folsomia candida* Willem exhibited decreases in horizontal movement and velocity when exposed to dimethoate-coated sand for 36 h.\(^\text{50}\) Pimentel et al.\(^\text{51}\) linked the reduction in mobility by insecticide-resistant *Rhyzopertha dominica* (Coleoptera: Bostrichidae) after sublethal doses of insecticide exposure to a trait aiding in the species’ physiological resistance, and thereby complicating pest management.

![Figure 2](image-url)  
**Figure 2.** Flight capacity of adult *H. halys* in 0–15 min, 15–30 min, 0.5–1 h and 1–2 h intervals after a limited 5-min exposure to insecticides when tethered on a flight mill system. Most of the flight happens within the first 15 min of time spent on the flight mill.
pheromone (e.g. for >24 h), while keeping them restricted to the site (moving <1 m away). This strongly suggests that attractive semiochemicals and pheromones may be a good means to ensure that H. halys takes up a lethal dose of insecticide through repeated contact with insecticide-coated surfaces. In other words, the reliability of retention of H. halys increases with the deployment of attractive semiochemicals, and as retention increases, so too does the probability of acquiring a lethal dose of insecticide.

This has ramifications for other management strategies as well. Adults exposed to sublethal doses of insecticides may disperse to untreated crops or areas. Because these individuals may have only received a sublethal dose of insecticide, they may still be mobile and able to cause economic damage. Indeed, as Mazzi and Dom35 state in a prior review, this emigration behavior may affect subsequent pest management decisions. H. halys is primarily a perimeter-driven pest,30,32 and as a result, border sprays can provide highly effective management.31 More recently, using provisional cumulative thresholds to trigger sprays for H. halys has shown great promise in providing control of pest populations while reducing insecticide usage (Short BD and Leskey TC, unpublished). Our results highlight the need for an efficacious residue present when using these strategies, particularly considering the short residual activity of many materials.18

However, for all of these management tactics, monitoring plays a crucial role in establishing the efficacy of treatment and the extent of the threat posed by H. halys in a particular field owing to their mobility. Over the last several years, there have been tremendous strides in developing an effective trap for monitoring H. halys. A large black pyramid trap seems most efficacious,32 though other trap designs also now seem to be effective options that are easier to deploy.35 The aggregation pheromone for H. halys has been identified,56 which has been found to have a synergistic effect on attraction of H. halys when combined with methyl decatrienoate, the pheromone from Plautia stali.57 The aggregation pheromone does not need to be highly purified,58 and these stimuli are effective at trapping H. halys throughout its introduced range in the United States.59 Thus, there is an effective trap and lure for helping to inform the efficacy of a treatment and the pressure of H. halys in agricultural landscapes, helping to alleviate the risk from mobile adults that are exposed to sublethal doses of insecticide.

Our research highlights the unintended biological effects that using insecticides may have for H. halys, and how this may affect the pest management tactics used. Even though we have employed the most efficacious compounds against H. halys, we were unable to eliminate the threat posed by H. halys, as adults remained mobile after brief exposure to field recommended rates. Further research should evaluate any sublethal effects that may affect recently identified generalist natural enemies60 or parasites61 of H. halys. In addition, a larger selection of insecticides (including those that are less effective) should be assessed for the effects of brief exposures on the mobility and behavior of H. halys, as well as investigating additional measures such as the consequences that brief doses may have on the fecundity and development of earlier developmental stages.

ACKNOWLEDGEMENTS

The authors would like to thank Morgan Douglas, John Cullum and Nate Brandt for their excellent technical assistance in the laboratory and field. This research was funded, in part, by a USDA-NIFA SCRI CAP Grant No. 2011-51181-30937. Mention of trade names or commercial products in this publication is solely for the purpose of providing scientific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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