

Discovery of Three Kairomones in Relation to Trap and Lure Development for Spotted Lanternfly (Hemiptera: Fulgoridae)

Miriam F. Cooperband,^{1,6,*} Jacob Wickham,² Kaitlin Cleary,^{1,3}
Sven-Erik Spichiger,⁴ Longwa Zhang,⁵ John Baker,⁴ Isaiah Canlas,^{1,3}
Nathan Derstine,^{1,3} and Daniel Carrillo³

¹Otis Laboratory, USDA APHIS PPQ S&T, 1398 W. Truck Road, Buzzards Bay, MA 02542, ²Chemical Ecology of Forest Insects Group, Institute of Zoology, Chinese Academy of Sciences, Beijing, China, ³Tropical Research and Education Center, University of Florida, 18905-SW-280th Street, Homestead, FL 33031, ⁴Pennsylvania Department of Agriculture, Bureau of Plant Industry, 2301 North Cameron Street, Harrisburg, PA 17110, ⁵Anhui Agricultural University, School of Forestry, Hefei, Anhui Province, China, and ⁶Corresponding author, e-mail: miriam.f.cooperband@aphis.usda.gov

Subject Editor: Brian Sullivan

Received 29 August 2018; Editorial decision 11 December 2018

Abstract

The spotted lanternfly, *Lycorma delicatula* (White), is an invasive phloem feeder recently introduced into North America that attacks a broad range of woody plants. When feeding in large numbers, they can seriously damage or kill a tree. Their preferred host is the invasive tree-of-heaven, *Ailanthus altissima* Swingle (Sapindales: Simaroubaceae), but they are serious pests of grape, *Vitis vinifera* L. (Vitales: Vitaceae) and a number of other commercially important host plants. Volatile collections were conducted on tree-of-heaven and grape, and the most abundant compounds from these plants present in samples and indicated in the literature were tested for attraction in the laboratory and field. Three compounds, methyl salicylate, (Z)-3-hexenol, and (E,E)- α -farnesene, were found to be highly attractive in laboratory behavioral bioassays. Methyl salicylate was attractive to all stages of *L. delicatula*, whereas the youngest nymphs were not as attracted to (Z)-3-hexenol or (E,E)- α -farnesene in laboratory bioassays. When comparing individual compounds, methyl salicylate attracted the most *L. delicatula*. Methyl salicylate lures in the field produced a two- to four-fold increase in captures compared with unbaited controls, and field testing also revealed a significant positive dose response. Of the several types of sticky bands tested, Web-Cote Industries sticky bands were found to be most efficient at trapping *L. delicatula* adults and nymphs.

Key words: chemical ecology, behavior, traps and lures, invasive species, detection

A newly introduced isolated population of *Lycorma delicatula* (White), spotted lanternfly, was found in Berks County, Pennsylvania, in late autumn of 2014 (Barringer et al. 2015) and the Pennsylvania Department of Agriculture (PDA) quickly established a quarantine in that county. In the subsequent three years, the population grew exponentially resulting in high densities of *L. delicatula*. By July 2018, the population had expanded to 13 counties in Pennsylvania and one county in Virginia (PDA 2018, VDACS 2018). Native to China, *L. delicatula* has a very broad host range, multiplies rapidly, and phloem feeds in large numbers mostly upon woody plants (Dara et al. 2015). *Lycorma delicatula* was first reported in Korea in 2006 where it has become a major pest of grape (Kim et al. 2011) and was later introduced into Japan (Kim et al. 2013, Tomisawa et al. 2013). Heavy feeding can result in branch dieback and even death of trees (M. Cooperband,

personal observation). Thick deposits of honeydew on foliage result in sooty mold growth, which interferes with photosynthesis and contaminates produce (Kim et al. 2011). Feeding can stunt grape vine growth, and the direct and indirect damage from feeding can cause nearly complete crop loss (Song 2010). They feed on over 70 host plant species (Dara et al. 2015), and heavy feeding is associated with wilt and dieback in *Ailanthus*, *Acer*, *Juglans*, *Humulus*, *Salix*, *Vitis*, and other species in Pennsylvania. They have a strong preference for tree-of-heaven *Ailanthus altissima* (Barringer et al. 2015), also native to China. *Ailanthus altissima* was introduced into the United States in 1784 as an ornamental and has become invasive and widespread throughout much of the United States (Snyder et al. 2013), particularly in disturbed areas, such as near railway tracks, highways, and quarries, creating a hospitable place for *L. delicatula* to establish once introduced.

It is unknown exactly how far *L. delicatula* are able to disperse naturally. However, in rural Pennsylvania, the quarantine expanded from the initial infestation by approximately 17 km between 2015 and 2016 (Parra et al. 2017). Human-assisted movement likely plays a major role in the spread of this species through inadvertent transport of their cryptic egg masses. *Lycorma delicatula* females have been observed to deposit egg masses on various outdoor objects such as tree trunks, stone, wooden posts, buildings, vehicles, wooden pallets, and a variety of other surfaces. Egg masses resemble a smudge of mud. Transport of *L. delicatula* to the United States most likely occurred as overwintering egg masses on imported goods from Asia. The abundance of their preferred host plant, *A. altissima*, in high traffic areas in the United States increases the likelihood of further spread and establishment.

Due to its status as a serious invasive pest in Korea, the Pennsylvania Department of Agriculture and United States Department of Agriculture moved rapidly to investigate what could be done to contain the population (Cooperband et al. 2018). It was determined that few practical tools existed for survey and detection of *L. delicatula*. At the time of its introduction, we were aware of no lures and only one trap developed for this insect (brown sticky paper bands, Korea Beneficial Insects Lab Co., Ltd., Ansong, Korea). A few months after its discovery, we commenced laboratory and field research hoping to develop new tools for detection of this species, such as lures and improved traps. Here, we describe our first attempts, starting a month prior to spring emergence of *L. delicatula* in 2015, to identify promising compounds for field tests and the follow-up studies that stemmed from these first attempts.

Methods

Volatile Collections

Volatile collections and literature reviews were used to determine which compounds dominated host plant volatile profiles, and these compounds were selected for purchase and use in behavioral bioassays and field studies. In 2015, domestic grape (*V. vinifera*) and tree-of-heaven (*A. altissima*) (collected from western Pennsylvania and southeastern Massachusetts, respectively) were potted and maintained in the greenhouse at the Otis Laboratory. Main stem diameters ranged between 1 and 2 cm at the base. Volatiles collections from these plants (performed soon after leaf-out but prior to insect emergence) were examined to identify dominant compounds, because in some insect–host systems, the dominant compounds can be key attractive kairomones (Miller and Rabaglia 2009). Twigs with a few leaves were cut off of each species and inserted into a tube containing hydroponic solution (Maxigrow, General Hydroponics, Sebastopol, CA), then placed in a sealed glass jar (0.5 liter) under full-spectrum lighting (60W compact fluorescent full spectrum light bulb, 5500K; Alzo Digital, Bethel, CT). Compressed air was cleaned with activated carbon filters and then passed through the sample jar at ~1 liter/min. The odor-laden air then passed through a volatile collection trap containing ~20 mg Hasyesep-Q (Hayes Separations, Inc., Bandera, TX) packed between small glass wool plugs inside a Pasteur pipette. Plant volatiles were collected for about 90 h with full spectrum lighting, after which traps were eluted with 0.2-ml hexane. The contents of the volatile collections were analyzed by injecting 1.0 μ l of elution into an Agilent 7890B gas chromatograph (GC) coupled with an Agilent 5977A mass spectroscopic detector. An Agilent DB-5 column (30 m, 0.32 mm I.D., 0.25 μ m film) was used with helium carrier gas and a 250°C inlet run in splitless mode. Oven temperature program was 50°C for 0.75 min, 10°C/min to

250°C held 30 min. Compounds of interest were identified by mass spectral matches to the NIST mass spectral library (v11, Agilent Technologies, Santa Clara, CA), and comparison of mass spectra to those in the literature and synthetic standards (Sigma Aldrich, St. Louis, MO and Bedoukian Research, Inc., Danbury, CT). Retention times of unknowns were also matched to synthetic standards. Relative abundance was quantified approximately by using peak areas of the total ion chromatogram.

Insects

The first laboratory studies commenced in 2015 with nymphs hatched from artificially warmed egg masses collected prior to field emergence. Egg masses were field collected in February 2015 following a freeze in Berks County, PA. Some egg masses were placed in an environmental chamber at 25°C with 16:8 (L:D) h under 40 W Gro-Lux wide-spectrum fluorescent bulbs (Sylvania, Danvers, MA). The remaining were kept at 10°C, then every few weeks some egg masses were transferred to the 25°C chamber in order to stagger early emergence and have a steady supply of nymphs for laboratory studies. Following emergence in the field, insects used in laboratory studies were field collected. Each stage was collected on the week it first appeared in the field in Berks County, PA. Insects were transported to the Otis Laboratory Insect Containment Facility in accordance with conditions set on permits from USDA and PDA (permit numbers P526P-15-00152 and PP3-0123-2015, respectively). Insects were maintained in screen cages (30 × 30 × 30 cm, Bugdorm, Megaview Science Co., Ltd., Taichung City, Taiwan) under broad-spectrum lighting in a walk-in environmental chamber at 25 ± 0.3°C with 16:8 (L:D) h, as described above. In 2015, it was not known what the best food source would be, so they were provided with both a potted *V. vinifera* plant and cuttings of *A. altissima* inside flower tubes containing hydroponic solution (Maxigrow) prepared according to the label instructions. Flower tubes with cuttings were replaced three times per week. Feeding activity was observed on both species until third instar, when all the nymphs on the potted *V. vinifera* abruptly moved to the *A. altissima* cuttings. After that, we provided them only with *A. altissima* cuttings. Cuttings quickly became depleted and wilted due to heavy *L. delicatula* feeding and required replacement at least every other day. High mortality usually occurred when cuttings were not replaced over weekends.

Since adults occur for ~2.5 mo, and are presumably engaged in different behaviors at different degrees of maturity (i.e., feeding, mating, and oviposition), we characterized adults into three stages: early, mid, or late. Early adults began with adult emergence and continued through populations on *A. altissima* trunks becoming mostly female, an interval lasting for ~5 wk. Mid-adults started when populations became even mixtures of males and females again prior to the first observation of egg masses, lasting about 3 wk. Late adults started when the first egg masses were observed and ended when the population died, usually the first frost.

Laboratory Bioassays

Laboratory bioassays were conducted within the Otis Laboratory Insect Containment Facility at 22 ± 0.3°C and 44 ± 1.8% RH. Custom Teflon Y-plate olfactometer behavioral bioassays were used to test *L. delicatula* for attraction to different compounds. For first-, second-, and third-instar *L. delicatula*, small Y-plates were used (16.5-cm long × 12.7-cm wide × 1.3-cm tall, with 1.9-cm wide channel; for details, see Cooperband et al. 2017). For fourth instars and adults, large custom Y-plates were used (28.6-cm long × 21.6-cm wide × 3.8-cm tall, with 5.1-cm wide channel). On the top and

bottom of the Teflon Y-plates, clear disposable transparency film (Apollo, Lincolnshire, IL) was affixed with electrode gel to form a seal (Spectra 360, Fairfield, NJ), then discarded at the end of each set. Air from an oil-free air compressor (small plates) or an air pump (Air Cadet, Thermo Scientific, Barrington, IL) (large plates) was passed through activated carbon filters, bubbled through water, and regulated with a flow meter (Analytical Research Systems, Gainesville, FL) before being split and directed through Teflon tubing into the Y-plate olfactometer. Air speed in the small and large Y-plates ranged between 30 and 35 and 23 and 24 cm/s, respectively. Tests were conducted between 0900 and 1500 hours under four wide-spectrum fluorescent bulbs running the length of the assay (Gro-Lux wide-spectrum 40 W). Behavioral bioassays were conducted on all stages of *L. delicatula* except for second instars, which were not available at the time of testing.

In the small Y-plates, the test odor was placed inside a 50-ml glass Erlenmeyer flask with a ground glass joint connected to one upwind arm of the Y with 0.64 cm diam. Teflon tubing and the same arrangement on the control arm received no odors. Air entered the large Y-plate bioassay through two 1.7-cm diam. Teflon tubes attached to the upwind arms, in which test odors were directly placed. Microcentrifuge tubes (0.25-ml polyethylene tubes, Thermo Fisher Scientific, Waltham, MA) each received a single 1 mm pinhole in the lid to facilitate odor release and then either 1 μ l of the test materials methyl salicylate (>99% purity, Sigma Aldrich),

(Z)-3-hexenol (>98% purity, Sigma Aldrich), or (*E,E*)- α -farnesene (84% purity, Bedoukian Research), or remained empty (control). A microtube with test material was then compared with an empty microtube. A clean Y-plate was used at the beginning of each bioassay session, and the first few insects in each session were tested with blank controls on both sides to ensure that there were no directional biases due to contamination, visual cues, or other factors. Then, the empty and test microtubes were inserted into the olfactometer upwind chambers for continued testing, and sides were alternated between sessions. Each session consisted of up to 20 replicates, depending on insect availability.

Insects were removed from rearing cages and held individually in vials away from host odors for approximately 30–60 min prior to their use in behavioral bioassays. An individual insect was released from a holding tube into the downwind end of the Y-plate and allowed 3 min to respond. A response was recorded when an insect traveled at least halfway up one of the arms of the Y, at which point the insect was removed. If no choice was made within 3 min, it was recorded as no response.

Lures and Release Rates

Lures were either made in-house or provided by various companies for field testing (Fig. 1). In addition to field testing, release rates were evaluated under laboratory conditions by calculating weight loss of lures over time. Lures were placed in a laboratory hood at 22–23°C

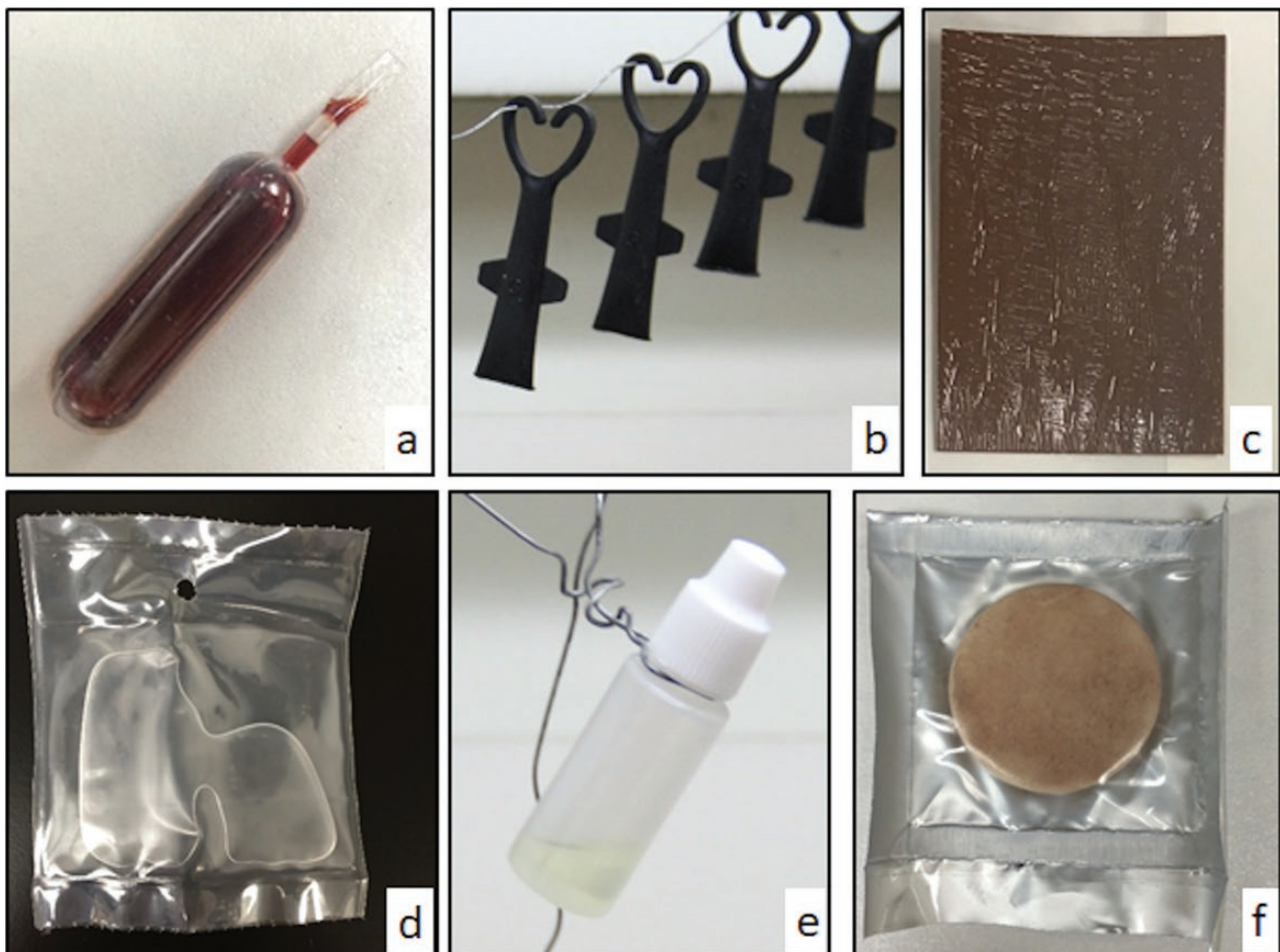


Fig. 1. Types of lures used in field studies: a) Alpha Scents pipette bulb, b) SinoGreen black heart, c) Hercon laminated square, d) Scentry pouch, e) SinoGreen LDPE bottle, and f) Alpha Scents high release.

and weighed up to three times per week. Loss of weight was attributed to loss of volatile compounds contained within the lures. After a period of weight loss, stable weight was an indication that the lure was spent. Release rates were not determined for the first two field experiments which were more preliminary in nature.

Field Studies

The first field study tested compounds found to be of interest based on laboratory studies prior to first emergence. As seasons progressed, laboratory and field studies continued to be staggered in this way, and results immediately guided the course of subsequent tests. The field studies are summarized in [Table 1](#).

Field studies took place during the summer of 2015 within the quarantine zone in Berks County, Pennsylvania, and during the summers of 2016 and 2017 in three locations in China, one site in Anhui and two sites in Beijing. In Pennsylvania, sticky bands were placed on *A. altissima* trees, unless otherwise indicated, and blocked by property. Lures were stuck or hung onto the sticky bands near the upper edge. In Beijing, all sticky bands were set on *A. altissima* street trees evenly spaced 5 m apart in several rows on the campus of Beijing Forestry University (BFU) (Qinghua E Rd.), or the Institute of Zoology (IOZ) (Lincui E Rd.) in two straight lines along a street. These two urban sites in Beijing were ~3.2 km apart, and each site was ~0.5 ha. Trees were grouped into blocks based on proximity, background populations, and other spatial considerations. Roughly 1,800 km to the south in Anhui (near Guanshan Rd. Feixi, Hefei), bands were placed on chinaberry *Melia azedarach* L. (Sapindales: Meliaceae) trees evenly spaced about 2 m apart in a plantation of roughly 0.5 ha. This site was selected after adult populations of *L. delicatula* were found feeding on the *M. azedarach* in 2015. A randomized complete block design was used in field studies, except one study with unequal sample sizes which had a completely randomized design, and two paired studies which had a matched pairs design. In 2016, the date each stage was first observed in the field was recorded in each of the three field locations (Berks County, Beijing, and Anhui), which were at three different latitudes.

Sticky tree bands were placed at breast height around trunks of similarly sized trees at each site. Average tree circumference among 90 Beijing trees was 83.3 (± 1.7) cm. Each study targeted a specific stage of *L. delicatula*. Therefore, bands were placed soon after a stage was first observed in the field and left for ~2 wk to target that particular stage. At the end of that time, bands were removed and tallied.

Field Experiment 1: Traps and Lures Targeting First and Second Instars

In Pennsylvania in 2015, five lures were selected for the first field test based on preliminary laboratory results, the literature, and availability. Spearmint oil was reportedly used in Korea as an *L. delicatula* attractant ([Moon et al. 2011](#)). The green-leaf volatile (Z)-3-hexenol was found in volatile collections and was attractive in preliminary laboratory bioassays, and in the literature was a major volatile component (28.7%) emitted by *A. altissima* ([Mastelic and Jerkovic 2002](#)). Although (Z)-3-hexenyl acetate and β -caryophyllene had not been otherwise tested, they were present in host plant volatile profiles, are known kairomones of other generalists, were readily available, and were reported to occur in *Ailanthus* profiles at 15.5 and 34.4%, respectively ([Mastelic and Jerkovic 2002](#), [El Ayeub-Zakhama et al. 2014](#)). In addition, a lure in development by Alpha Scents (West Linn, OR) for the brown marmorated stink bug

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), an invasive generalist hemipteran with similar feeding preferences as *L. delicatula* that include host *A. altissima* ([Haye et al. 2015](#)), was tested. A trapping study was conducted using ten private properties (spaced from 0.6 to 3.1 km apart from each other) and tree species as blocks, where each property had a cluster of seven similarly sized trees of the same species (trees in clusters were spaced less than 7 m apart), and each tree was assigned one of seven treatments. Tree species other than *Ailanthus* were included because PDA found high numbers of *L. delicatula* on these tree species, and it was difficult at the time to find enough *A. altissima* trees in the limited area of infestation in clusters of seven trees. The seven treatments had lure and trap combinations as follows: six treatments on brown paper sticky bands (0.2-m wide; Korea Beneficial Insects Lab Co. [KBIL], Ltd., Ansong, Korea) were as follows: 1) spearmint oil, 2) (Z)-3-hexenol, 3) (Z)-3-hexenyl acetate, 4) a blend of (Z)-3-hexenol + (Z)-3-hexenyl acetate + β -caryophyllene (2:1:1), 5) an Alpha Scents five-component blend (equal parts (Z)-3-hexenol, nonanal, β -caryophyllene, linalool, and Alpha Scents Enriched Ginger Oil with 8% α -copaene), 6) blank control, and the final treatment was 7) an additional blank control using clear sticky bands (Clear Roll Trap - No-Mess Adhesive; Alpha Scents) aimed at testing a different glue. Each lure consisted of a transfer pipet bulb containing 4 ml of material (Alpha Scents; [Fig. 1a](#)). One block each of trees were *Acer rubrum* L. (Sapindales: Sapindaceae), *Tsuga canadensis* (L.) Carr. (Pinales: Pinaceae), *Sassafras albidum* (Nuttall) (Laurales: Lauraceae), and *Paulownia tomentosa* (Thunb.) (Lamiales: Paulowniaceae); three blocks used *Liriodendron tulipifera* L. (Magnoliales: Magnoliaceae), and three blocks used *A. altissima*. As a result, each treatment had 10 replicates. The study took place from 3 June to 7 July 2015. The first 2 wk of trapping targeted first instars, then the sticky bands were replaced with fresh bands, and lures were reused for an additional 2 wk (4 wk total use) targeting the second instars.

Field Experiment 2: Traps and Lures Targeting Adults

In PA in 2015, when fourth instars and adults emerged, it was soon discovered that the KBIL bands were not able to capture them, and it was observed that large numbers of *L. delicatula* would amass on tree trunks below the KBIL bands, but they avoided walking on or being captured by the bands. A better trapping tool was urgently needed that could capture adults in order to assess differences between lures, and there was no time for preliminary testing. An excess of traps designed for *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) emerald ash borer (EAB), purple prism traps (Great Lakes IPM, Vestaburg, MI; [Francese et al. 2008, 2010](#)), was in our possession, so they were modified and tested against *L. delicatula*. A study was conducted across an area approximately 5.3-km long and 1.3-km wide that included seven blocks (on average 0.2 ha, and 0.4–2 km from each other). Each replicate had a cluster of 10 *A. altissima* trees (most were within 7 m from a neighboring tree in the cluster), 5 of which received a KBIL sticky band, and the other 5 received the EAB purple prism trap wrapped around the tree and taped to the trunk at the bottom and top with paper packing tape (traps were matched pairs; [Fig. 2](#)). Each of the five traps received one of the five lure treatments for a randomized complete block design (each lure was a pipette bulb containing 4 ml of material; Alpha Scents, West Linn, OR): 1) control (no lure), 2) mixed farnesene isomers, 3) methyl salicylate, 4) (Z)-3-hexenol, or 5) methyl salicylate + (Z)-3-hexenol. All compounds were from Sigma Aldrich. This study was conducted from 26 August to 9 September 2015.

Table 1. Overview of main field studies conducted in 2015, 2016, and 2017 in Pennsylvania and China

| Experiment | Year | Dates | Location | Stage | Design, treatments | Trap(s) | Lure type(s) | Contents of lure(s) ^a | Per lure (ml) | Laboratory release rates (mg/d) | N |
|------------|------|----------------------------------|----------|-------------------------------|--|------------------------------|--|--|--|---------------------------------|---------------------------|
| 1 | 2015 | June 3–June 17 June 17–July 7 | PA | First instar Second instar | RCB, 7 treatments: Clear + no lure vs. KBIL + no lure vs. KBIL + lure (5) | KBIL, Alpha Scents Clear | None, Alpha Scents bulb | Control (Z)-3-hexenyl acetate Spearmint (Z)-3-hexenol 3-Blend 5-Blend Control Mixed farnesene isomers, (Z)-3-hexenol, Methyl salicylate, (Z)-3-hexenol + methyl salicylate Control Methyl salicylate | 0 4 4 4 4 0 4 2x4 | n.d. n.d. | 10 |
| 2 | | Aug. 26–Sept. 9 | PA | Early adults | RCB, MP, 10 treatments: 4 lures and control compared on pairs of KBIL bands and Purple prism traps | KBIL, Purple Prism | None, Alpha Scents bulb | Control Mixed farnesene isomers, (Z)-3-hexenol, Methyl salicylate, (Z)-3-hexenol + methyl salicylate Control Methyl salicylate | 0 4 4 4 4 0 4 2x4 | n.d. | 7 |
| 3 | 2016 | April 29–May 13 May 13–May 27 | Beijing | First instar Second instar | RCB, 4 treatments: 3 lures and control | KBIL | None, in-house bulb | Control Methyl salicylate | 0 2 4 | 0 16 16 32 | 10 |
| 4 | | Aug. 26–Sept. 10 | Beijing | Mid adults | RCB, 3 treatments: 3 unbaited traps | Packing tape, KBIL, Web-Cote | None | — | — | — | 12 |
| 4 | 2017 | April 19–May 2 | Anhui | First instar | RCB, 3 treatments: 3 baited traps | Packing tape, KBIL, Web-Cote | Hercon squares (x2) | Methyl salicylate | n.d. | 49 | 13 |
| 4 | | May 2–May 16 | Anhui | Second instar | RCB, 3 treatments: 3 unbaited traps | Packing tape, KBIL, Web-Cote | None | — | — | — | 13 |
| 5 | | May 16–June 2 | Anhui | Third instar | CR, 4 treatments: 3 release rates and control | Web-Cote | None, Alpha Scents bulb (x1), SinoGreen black hearts (x2), Hercon squares (x2) | Methyl salicylate | n.d. | 0 17 39 46 11 | 6 13 13 13 10 |
| 6 | | June 8–June 21 | Beijing | Third instar | RCB, 2 treatments: lure and control | Web-Cote | None, Scentry pouch | (Z)-3-hexenol | n.d. | — | 10 |
| 7 | | June 13–June 15 | Beijing | Fourth instar | RCB, 3 treatments: 2 release rates and control | Web-Cote | None, SinoGreen bottle (x3), SinoGreen bottle (x6) | (E,E)- α -farnesene | 0 1 ml x 3 1 ml x 6 n.d. | 0 17 35 0 53 | 15 10 |
| 8 | | July 20–July 29 | Beijing | Mid adults | MP, 2 treatments: lure and control | Web-Cote | None, Alpha Scents high-release | Methyl salicylate | n.d. | — | 10 |

Release rates reported for each lure were measured under laboratory conditions. It should be noted; however, that field release rates were likely higher due to higher temperatures and wind speeds.

(RCB) randomized complete block design; (CR) completely randomized design; (MP) matched pairs design; (PA) Pennsylvania; (n.d.) no data.

^aCompounds tested in lures had the following purities (if known): spearmint oil, purity not reported; Alpha Scents (Z)-3-hexenol, 98%; Alpha Scents (Z)-3-hexenyl acetate, 98.1%; Alpha Scents mixed farnesene isomers (purity not reported); Alpha Scents methyl salicylate, 95%; in-house methyl salicylate, >99% (Sigma Aldrich); Hercon methyl salicylate (purity not reported); Scentry (Z)-3-hexenol, purity not reported; SinoGreen methyl salicylate, >99%; (E,E)- α -farnesene (Bedoukian), 83.9%; Alpha Scents 3-Blend: (Z)-3-hexenol, 98%; (Z)-3-hexenyl acetate, 98.1%; β -caryophyllene, 98%; Alpha Scents 5-Blend: (Z)-3-hexenol, 98%; nonanal, 95%; β -caryophyllene, 98%; linalool, 96%; Alpha Scents Enriched Ginger Oil with 8% α -copaene (purity not reported).



Fig. 2. Purple prism traps shown wrapped around *A. altissima* trees and taped to trunks with packing tape and used for comparison to KBIL sticky bands.

Field Experiment 3: Dose–Response to Methyl Salicylate Targeting First and Second Instars

In Beijing in 2016, we tested three formulations using methyl salicylate in pipette bulb lures (formulated in-house) as well as blank controls, using KBIL sticky bands. The desired amount of methyl salicylate (>99% purity, Sigma Aldrich) was measured into a glass vial, then transferred to a low-density polyethylene disposable transfer pipet (thin stem, 4.25-ml capacity, Thermo Fisher Scientific). The pipet tip was folded and melted shut with a heat sealer. Four treatments of methyl salicylate were tested: blank control (0 mg/d), a pipet containing 2 ml (~16 mg/d), 4 ml (~16 mg/d), and two pipets each containing 4 ml (~37 mg/d). This study had 10 replicates and was conducted from 29 April to 27 May 2016.

Field Experiment 4: Comparison of Sticky Bands

Three sticky band traps consisting of generic packing tape, KBIL, and Web-Cote Industries Tree Band (TB50M-2159; Hamburg, NJ), were compared for captures of adults in Beijing (26 August to 10 September 2016). In Anhui in 2017, the same three types of bands were tested with first- and second-instar *L. delicatula* (from 19 April to 16 May 2017). For first instars, each band received two Hercon methyl salicylate laminated square lures (~49 mg/d). Bands targeting second instars and adults were tested without lures. There were 12 and 13 replicates for adults and nymphs, respectively.

Field Experiment 5: Release Rates of Methyl Salicylate

A comparison between commercial lures releasing different rates of methyl salicylate was conducted in Anhui, China, in 2017, targeting third-instar (16 May to 2 June) and fourth-instar (2 June to 16 June) *L. delicatula*. Traps were baited with either two Black Heart lures (Nanjing Xinan SinoGreen Biological Technology Co. Ltd., Nanjing, China; Fig. 1b), two Hercon squares (Fig. 1c), or one Alpha Scents pipette bulb. Average daily laboratory release rates for these treatments over 17 d were roughly 39, 46, and 17 mg/d, respectively. In order to maximize replication with limited available trees, treatments containing lures were replicated 13 times and unbaited

control traps were replicated 6 times in a completely randomized design. These treatments were tested using Web-Cote sticky bands on *M. azedarach* trees in a plantation, where trees were evenly spaced roughly 2 m apart in a monoculture.

Field Experiment 6: Lures Containing (Z)-3-Hexenol

Lures containing (Z)-3-hexenol (Scentry Biologicals, Inc., Billings, MT; Fig. 1d), with laboratory release rate of 11 mg/d, were compared with blank controls against third-instar *L. delicatula* in Beijing in 2017 using Web-Cote sticky tree bands. This study had 10 replicates and a randomized complete block design and was conducted from 8 June to 21 June 2017.

Field Experiment 7: Lures Containing (E,E)- α -Farnesene

Either three or six LDPE bottles (Fig. 1e; Nanjing Xinan SinoGreen Biological Technology) each containing 1 ml of (E,E)- α -farnesene, or blank controls were compared using Web-Cote tree bands in Beijing in 2017. The laboratory release rate of each bottle was 5.8 mg/d; thus, this study compared treatments of 17.4 mg/d, 34.8 mg/d, and blank controls. Because of what appeared to be strong tree effects, in which the numbers of *L. delicatula* were extremely high in some trees and low in adjacent similar trees, it was difficult to rule out or overcome these effects in some locations. Therefore, for this study, we employed a mark-release-recapture approach to test these lures using fourth instar nymphs. Each block contained three trees with a central point on the ground 3.5 m from each tree where 12 marked nymphs were released. Nymphs in adjacent blocks received different color fluorescent dyes (BioQuip Products, Inc. Rancho Dominguez, CA). Nymphs were collected on 12 June 2017 in Beijing and marked and released the following day. There were 15 replicates and traps were tallied after 48 h.

Field Experiment 8: Lures With High-Release Methyl Salicylate Targeting Adults

To test commercial high release methyl salicylate lures (~53 mg/d in the laboratory; Alpha Scents; Fig. 1f) in Beijing, China, in 2017, we again employed a mark-release-recapture approach to overcome tree effects. Ten pairs (replicates) of *A. altissima* trees were banded with Web-Cote, and in each pair, one tree had a lure and the other had none (matched pair design). Twelve *L. delicatula* adults marked with different dye colors corresponding to release point were released on the ground halfway between each pair of trees, their movement was monitored daily from 20 July to 29 July 2017, and traps were tallied after 9 d.

Statistical Analyses

Dual choice laboratory bioassays were analyzed using a chi-square test, with significance at $\alpha = 0.05$ when the *G*-test statistic was ≥ 3.841 (Rohlf and Sokal 1995, Sokal and Rohlf 1995). Means and SEs are reported for field data. Paired *t*-tests were performed on paired tree studies. When data satisfied Shapiro–Wilk’s test for normality and Welch’s test for equal variance (JMP v.10.0.0), field tests were analyzed using analysis of variance (ANOVA) and Tukey means separation with significance at $\alpha = 0.05$. Data that were not normal were rank transformed and analyzed in the same way. If transformation did not satisfy these criteria, nonparametric Wilcoxon test with a Bonferroni adjustment was used for multiple comparisons. Correlations between mean capture rates and dose were evaluated using a linear regression model (JMP v.10.0.0).

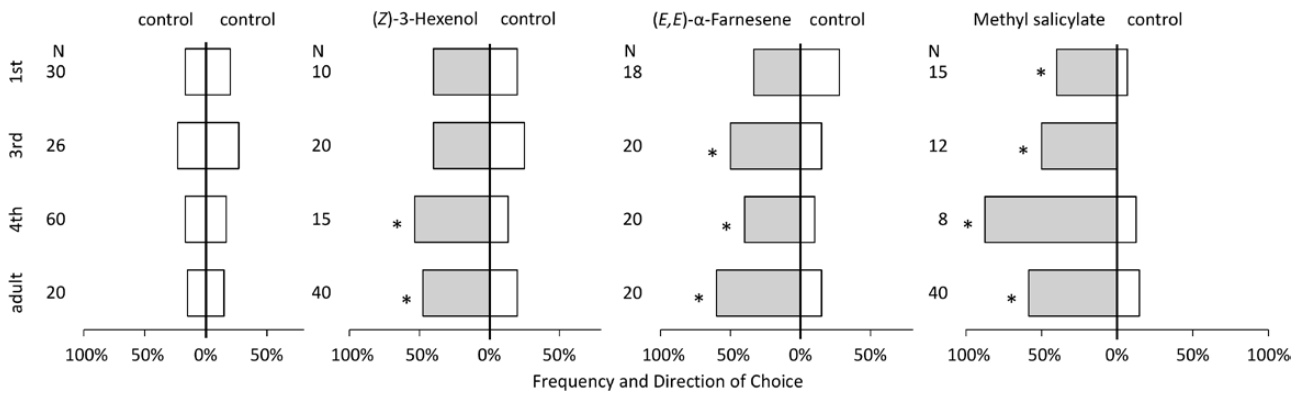


Fig. 3. Reactions to odors displayed by *L. delicatula* at different stages in laboratory dual-choice olfactometer (Y-plate) walking bioassays, in response to three compounds commonly found in volatiles from their preferred host, *A. altissima*. Asterisks indicate one side was chosen significantly more than the other (chi-square test, $\alpha = 0.05$).

Results

Volatile Collections

Volatile collections of *A. altissima* and *V. vinifera* grown in the greenhouse contained fewer than 10 compounds that composed >1% of the sample. Volatiles collected from the twigs and leaves of *A. altissima* were dominated by the compound (*E,E*)- α -farnesene, which constituted 78% of the material. In the literature, another dominant volatile emitted by *A. altissima*, which did not show up in our analysis, was reported to be (*Z*)-3-hexenol (28.7%; Mastelic and Jerkovic 2002). Similarities between grape and *A. altissima* volatiles, respectively, included (*E,E*)- α -farnesene (19.0 and 78.1%) and methyl salicylate (3.8 and 2.0%). Based on our findings and the literature, these three compounds were targeted for the early laboratory bioassays to evaluate attraction. In addition, (*Z*)-3-hexenyl acetate (Mastelic and Jerkovic 2002) and β -caryophyllene (El Ayeub-Zakhama et al. 2014) were reported to occur in *A. altissima* profiles at 15.5 and 34.4%, respectively, so these two compounds were included in the first field study.

Dual Choice Bioassays

All stages except second instars were tested in the Y-plate bioassays and offered a choice between an odorless control and either (*Z*)-3-hexenol, (*E,E*)- α -farnesene, or methyl salicylate (Fig. 3). Attraction to different compounds differed depending on the stage of the insect. First instars showed a significant preference for methyl salicylate, but not for the other two compounds. Third instars were attracted to methyl salicylate and (*E,E*)- α -farnesene, but not (*Z*)-3-hexenol. Fourth instars and adults were both significantly attracted to all three compounds tested. Controls with no odor on both sides demonstrated that there was no bias toward one side or the other in the behavioral bioassays, and response rate was greater when any of the three compounds was present.

Field Studies

First appearance of life stages of *L. delicatula* varied among the three different experimental sites, with developmental rates being apparently similar among them (Fig. 4). The date of first appearance of any particular life stage at the different sites generally corresponded to site latitude, with later appearance of the same life stages occurring at greater latitudes.

Field Experiment 1: Traps and Lures Targeting First and Second Instars

Number of *L. delicatula* caught per band per day in the first 2 wk of trapping (first instars) were significantly different among blocks

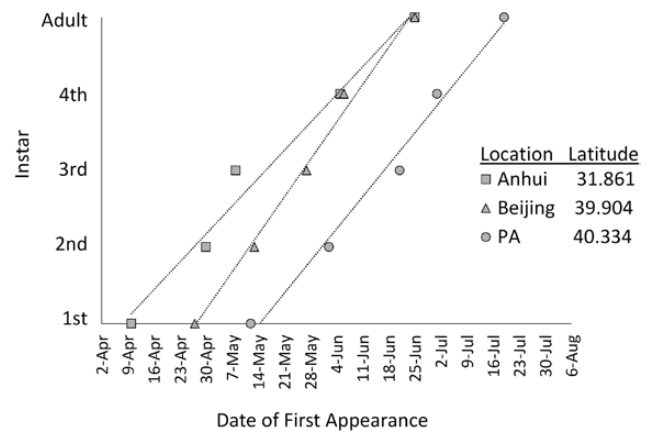


Fig. 4. Dates on which each stage of *L. delicatula* were first observed in each of the three field locations and latitudes. Lines represent linear regression for each population.

($F = 4.82$, $df = 9, 54$, $P < 0.001$) and among trap and lure treatments ($F = 2.63$, $df = 6, 54$, $P = 0.026$). Brown band traps baited with the 100% (*Z*)-3-hexenol lure caught significantly more first-instar *L. delicatula* than unbaited clear bands, but there were no other differences among treatments (Fig. 5). However, there was a positive correlation between mean catch per day of first instars and the relative amount of (*Z*)-3-hexenol in the lure ($R^2 = 0.92$, $P = 0.040$; Fig. 6). The same lure/trap treatments during weeks 3 and 4 (targeting second instars) were not significantly different ($F = 0.75$, $df = 6, 54$, $P = 0.613$), whereas blocks differed ($F = 6.28$, $df = 9, 54$, $P < 0.001$) (data not shown). The unbaited clear band caught the least *L. delicatula*.

Field Experiment 2: Traps and Lures Targeting Adults

Over the 2-wk trapping period, the purple prism traps caught significantly more *L. delicatula* adults than the KBIL bands ($t = 6.26$, $df = 34$, $P < 0.001$). ANOVAs on ranked data were conducted separately on each trap type to evaluate differences among lures. The number of *L. delicatula* adults caught on either KBIL bands or purple prism traps did not differ significantly among lure treatments (KBIL band: $F = 0.929$, $df = 4, 6$, $P = 0.464$; purple prism trap: $F = 1.859$, $df = 4, 6$, $P = 0.151$). However, on purple prism traps, the two treatments containing methyl salicylate caught the greatest number of adult *L. delicatula* (Fig. 7).

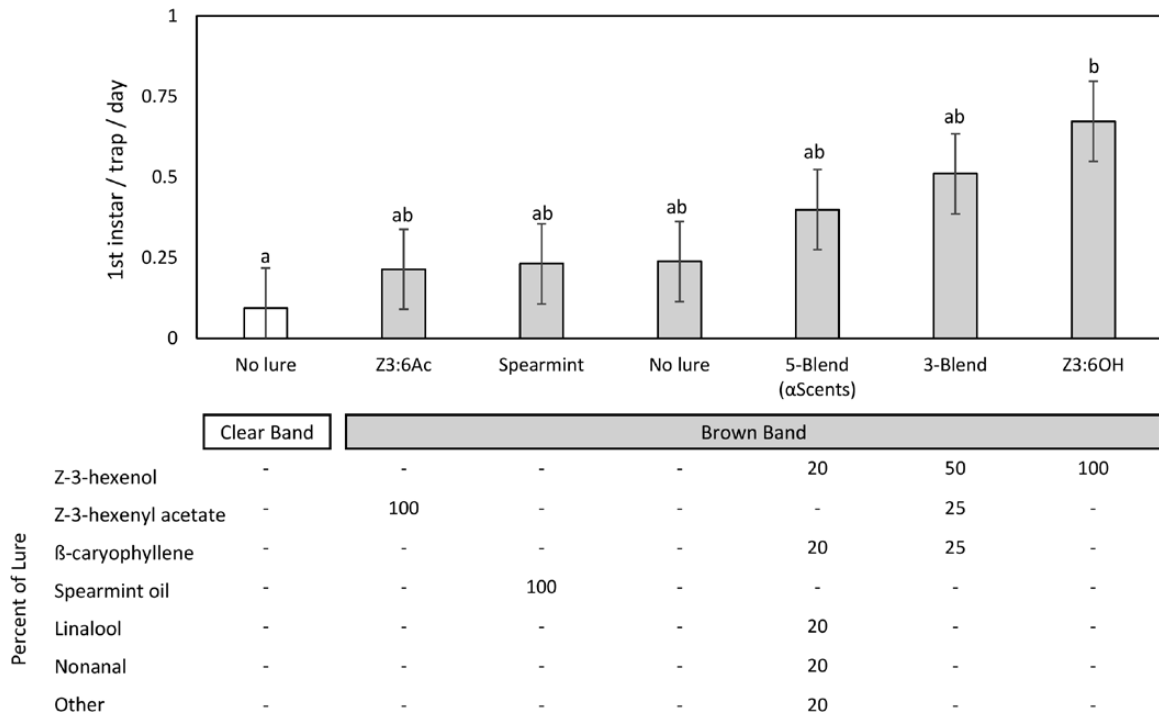


Fig. 5. Mean number (\pm SE) of first instar *L. delicatula* trapped per band per day in Pennsylvania in 2015 over a period of 2 wk using different traps and lures (ANOVA, $N = 10$). Pipette lures each had the same capacity (~ 4 ml), and lure contents are listed by percentage volume below each bar but does not equate to release rate of each compound, which was not measured. Columns that do not share the same letters are significantly different.-

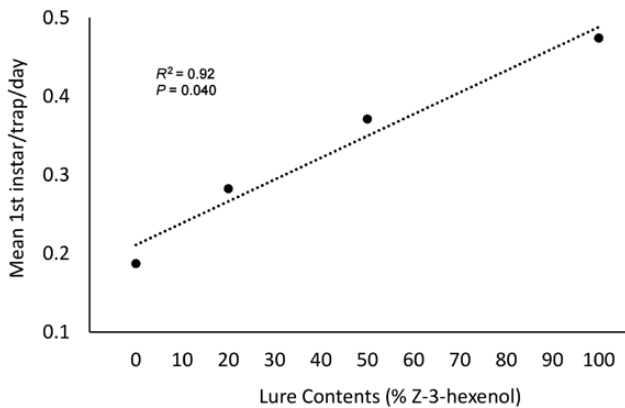


Fig. 6. Linear regression model showing positive correlation between mean number of first-instar spotted lanternfly, *L. delicatula*, caught per trap per day and the percent of (Z)-3-hexenol contained in lures.

Field Experiment 3: Dose–Response to Methyl Salicylate Targeting First and Second Instars

The release bulbs containing 2 and 4 ml of methyl salicylate both released roughly 16 mg/d, so those two treatments were combined for the analysis. Linear regressions were performed on number of first and second instars caught over two consecutive 2-wk intervals on KBIL sticky bands in Beijing in 2016. For first instars, the regression was not statistically significant ($R^2 = 0.023$, $P = 0.355$). However, second instars showed a significant dose response ($R^2 = 0.135$, $P = 0.020$; Fig. 8).

Field Experiment 4: Comparison of Sticky Bands

For first instars responding to traps baited with identical methyl salicylate lures, Web-Cote bands captured significantly more nymphs

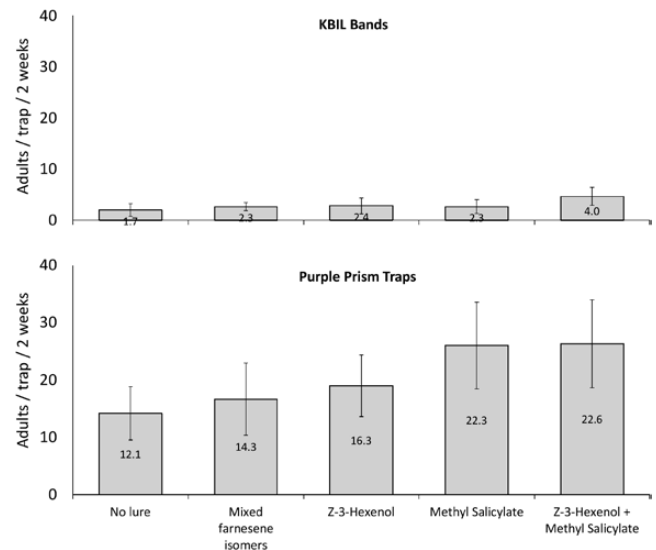


Fig. 7. Mean number (\pm SE) of adult *L. delicatula* trapped per band over a 2-wk period in Pennsylvania in 2015 using different traps and lures ($N = 7$). Purple prism traps caught significantly more adult *L. delicatula* than KBIL sticky bands (paired *t*-test). However, no significant differences were detected among lure treatments (ANOVA).

than KBIL bands ($Z = 3.43$, $N = 13$, $P < 0.001$) or packing tape ($Z = 4.31$, $P < 0.001$), and KBIL bands also caught significantly more nymphs than packing tape ($Z = 3.74$, $P < 0.001$) (Fig. 9). For second instars responding to unbaited traps, Web-Cote bands caught significantly more *L. delicatula* than the packing tape (Wilcoxon, $Z = 4.31$, $P < 0.001$) or KBIL bands ($Z = 2.80$, $P = 0.005$), but numbers captured by Web-Cote and KBIL were not significantly different using the Bonferroni adjusted $\alpha = 0.025$ ($Z = 2.23$, $P = 0.026$). Unbaited

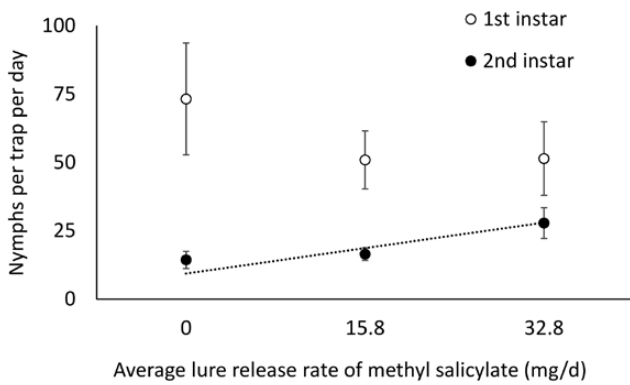


Fig. 8. Response of first- and second-instar spotted lanternfly, *L. delicatula*, to different doses of methyl salicylate in pipette bulb lures in Beijing, China, in 2016. No significant dose response was detected for first instars. However, a significant dose response was observed for second instar *L. delicatula* ($R^2 = 0.135$, $P = 0.020$).

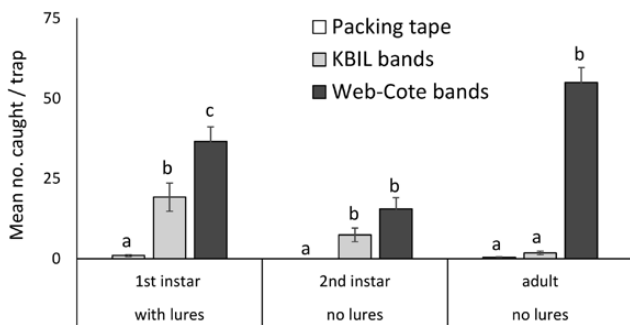


Fig. 9. Mean (\pm SE) number of *L. delicatula* caught per trap over 2-wk trapping periods in Anhui (in 2017 targeting first and second instar) and Beijing (in 2016 targeting adults). Methyl salicylate lures were deployed to all trees for first instars, but no lures were used for second instars and adults. For each stage, columns that do not share the same letters are significantly different (Wilcoxon for each pair).

Web-Cote bands captured more adults than KBIL bands (Wilcoxon test, $Z = 4.14$, $P < 0.001$) and packing tape ($Z = 4.21$, $P < 0.001$), and there was no difference between KBIL bands and packing tape using the Bonferroni adjusted $\alpha = 0.025$ ($Z = 2.25$, $P = 0.025$).

Field Experiment 5: Release Rates of Methyl Salicylate

A significant dose response was found when targeting third instar *L. delicatula* in Anhui in 2017 using different methyl salicylate lures and release rates on Web-Cote bands ($R^2 = 0.094$, $P = 0.041$; Fig. 10). The maximum release rate, 46 mg/d (as measured under laboratory conditions), caught the most *L. delicatula*, followed by the 39 mg/d treatment, the 17 mg/d treatment, and the blank control.

Field Experiment 6: Lures Containing (Z)-3-Hexenol

There was no significant difference between sticky bands baited with (Z)-3-hexenol lures and unbaited control sticky bands targeting third- and fourth-instar *L. delicatula* in Beijing in 2017 ($F = 1.029$, $df = 1, 18$, $P = 0.324$). However, there appeared to be a trend toward higher catches in the (Z)-3-hexenol baited bands, which captured an average of 17.4 (± 6.9) nymphs per tree, whereas controls captured 10.0 (± 2.5) nymphs per tree.

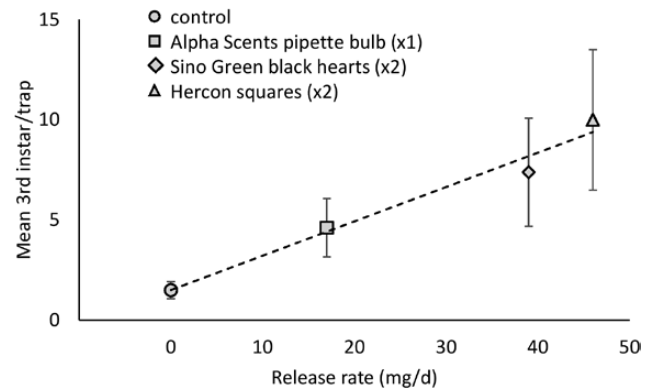


Fig. 10. Mean number (\pm SE) of third-instar spotted lanternfly, *L. delicatula*, captured per trap in Anhui, China, comparing response to lures containing methyl salicylate in a plantation of *M. azedarach*. A linear regression model showed a significant correlation between mean trap capture and release rate of lures used ($R^2 = 0.094$, $P = 0.041$).

Field Experiment 7: Lures Containing (E,E)- α -Farnesene

No significant dose response was found in Beijing, comparing (E,E)- α -farnesene inside LDPE bottle lures (0, 17 mg/d [3 bottles], or 35 mg/d [6 bottles]), for marked-recaptured *L. delicatula* ($R^2 = 0.004$; $P = 0.677$), or total marked and unmarked *L. delicatula* ($R^2 = 0.008$; $P = 0.557$) 48 h after marked insects were released. On average, the control, 3-bottle, and 6-bottle treatments recaptured 1.5 (± 0.38), 1.8 (± 0.38), and 1.2 (± 0.38) marked *L. delicatula*, respectively, over 48 h.

Field Experiment 8: Lures With High-Release Methyl Salicylate Targeting Adults

A paired *t*-test in Beijing revealed significantly more adult *L. delicatula*, both marked ($t = -3.73$, $df = 9$, $P = 0.002$) and total of marked and unmarked ($t = -4.43$, $df = 9$, $P = 0.001$), were captured on Web-Cote tree bands baited with methyl salicylate lures (which released 53 mg/d under laboratory conditions) compared with unbaited controls (Fig. 11).

Discussion

Through laboratory and field testing, we discovered three host plant volatiles that, in their synthetic form, were attractive to *L. delicatula* in the laboratory but produced mixed results in field studies. Evidence indicated that (E,E)- α -farnesene, methyl salicylate, and (Z)-3-hexenol were major volatiles associated with their preferred host plant, *A. altissima*. These three synthetic compounds, when tested in a dual choice olfactometer under laboratory conditions, were all significantly attractive to various stages of *L. delicatula* (Fig. 3). Interestingly, of these three compounds, first-instar *L. delicatula* were significantly attracted only to methyl salicylate in the bioassays but did appear to show attraction to (Z)-3-hexenol in the field (Fig. 6). Methyl salicylate is a common plant defensive compound, and (Z)-3-hexenol is a green-leaf volatile, both of which can be induced in some plants by insect feeding (Tholl et al. 2006). Their various roles have been studied in plant–insect, plant–plant, and insect–insect interactions (Tholl et al. 2006, Wei and Kang 2011). The sesquiterpene (E,E)- α -farnesene is a constituent in numerous plant and fruit odors and some insect pheromones (Boeve 1999, Prokopy et al. 2001, Sobotnik et al. 2008, Suckling et al. 2010).

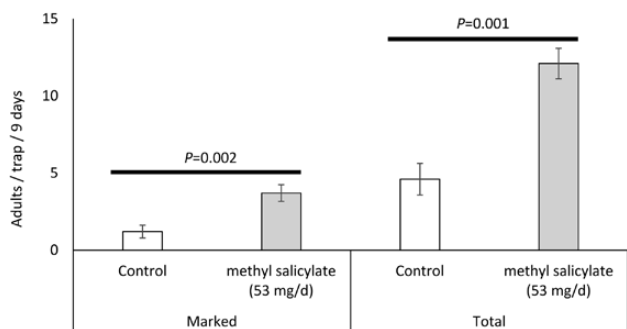


Fig. 11. Twelve adult spotted lanternfly, *L. delicatula*, were marked and released halfway between each pair of banded *A. altissima* trees, one baited with a methyl salicylate lure (Alpha Scents Hi Release) and the other with no lure (control). Significantly more marked adults (left) and total adults (right) were captured on trees with lures than control trees (paired *t*-test, $N = 10$).

Differences in volatile profiles of plants of the same species may be observed for a variety of reasons including differences due to genetic variation, soil, water, stress, nutrition, damage, plant part, and collection technique. For instance, [Albouchi et al. \(2013\)](#) air-dried *A. altissima* leaves for a week prior to extraction by hydrodistillation, whereas others used fresh plant material. In [Mastelic and Jerkovic \(2002\)](#), volatiles were collected from 2- to 20-yr-old *A. altissima* trees in September, whereas our volatiles were collected from estimated 5-yr-old *A. altissima* trees in the early spring just after leafing out. Tree age or the time of year samples are taken may be relevant, since *A. altissima* is deciduous and its seasonality is matched by the seasonal biology of *L. delicatula*. [El Ayeb-Zakhama et al. \(2014\)](#) reported on the volatile profiles of different tissues of 20-yr-old *A. altissima* trees and described differences in volatiles produced from flowers, leaves, roots, stems, and samaras. The above studies used hydrodistillation techniques to collect volatiles. Hydrodistillation is a process that uses heat to extract volatiles. However, headspace volatile collection of living plant material at ambient temperatures and under lighting to promote photosynthesis can provide a more representative profile of the volatiles emitted by the living plant ([Tholl et al. 2006](#)).

Field studies with lower release rate lures were inconclusive, but suggested trends of attraction to the three compounds ([Figs. 5–8](#)). Follow-up field studies using higher doses and dose–response tests revealed that methyl salicylate was the most successful of the lures we tested, and the highest dose of methyl salicylate we tested attracted the most third instar *L. delicatula* ([Fig. 10](#)). A methyl salicylate lure with an even higher release rate was used to successfully trap adult *L. delicatula* ([Fig. 11](#)). The highest release rate lures caught approximately threefold the number of *L. delicatula* as compared with unbaited controls.

Our first field study included tests of lures containing (Z)-3-hexenyl acetate; spearmint oil; a blend of (Z)-3-hexenol + β -caryophyllene + (Z)-3-hexenyl acetate; or a blend of (Z)-3-hexenol, β -caryophyllene, linalool, nonanal, and a plant essential oil containing 8% α -copaene ([Fig. 5](#)). Spearmint oil lures were field tested, because spearmint oil was documented as a strong attractant to *L. delicatula* in the literature ([Moon et al. 2011](#)). However, the spearmint lures we tested caught numbers similar to the unbaited controls. Although linalool has been reported as a repellent for *L. delicatula* ([Yoon et al. 2011](#)), the blend containing 20% linalool did not significantly reduce *L. delicatula* catch compared with other treatments.

Although some attraction was seen with (Z)-3-hexenol and (E,E)- α -farnesene in both the laboratory and the field ([Figs. 3, 5, 6, and 7](#)), the lures we tested did not result in reliable attraction to

these compounds in the field (field studies 6 and 7). Perhaps, the amounts of these two compounds released by the lures we tested cannot compete with the amounts released by host plants in the field. Improving lure performance may depend upon further development of lures and research optimizing release rates for these two compounds. Methyl salicylate, being moderately attractive, inexpensive, commercially available, and having chemical properties that allowed for steady and high release rates over time through a membrane, facilitated its development into a lure.

A major impediment in early field testing for attraction to lures was trap-related, as the KBIL sticky tree bands were able to capture only younger nymphs effectively. Older nymphs increasingly escaped or evaded capture by KBIL bands, and adults, although observed in high abundance next to the bands, were either not walking onto them or escaped being captured. One possible explanation is that they may test surfaces prior to walking on them and tend to avoid the glue. Additionally, as they increase in size, they also increase in strength and are better able to push themselves out of the glue. By the middle of July in 2015, it was clear that we would not be able to continue testing attractants in the field without improved trap designs, and there was little time to test modified trap technology in order to test lures. Fortunately, the purple prism traps typically used against *A. planipennis* were significantly more effective than the KBIL sticky bands for adult *L. delicatula*. A study in Korea comparing brown, yellow, and blue sticky bands found that the brown sticky bands performed significantly better, but the authors did not report wavelengths or if there were differences in glue composition ([Choi et al. 2012](#)). Another study found that *L. delicatula* preferred ultraviolet over white light, and blue over white, yellow, or green light ([Jang et al. 2013](#)). Apart from color, the fact that the prism traps were approximately three times longer than KBIL bands, had a larger surface area, and were coated with a thicker glue could have influenced their enhanced effectiveness. Most adults were caught by the wings in the middle of the EAB purple prism traps, rather than along the bottom margin where nymphs are always caught, suggesting that they were intercepted during flight rather than while walking. Although they improved adult trap catch, they were difficult to wrap around trees and not practical for use in any large-scale trapping program. The Web-Cote sticky bands were subsequently tested on first and second instars and adults, and they were found to catch significantly more first instars and adults than the KBIL bands. Although not statistically significant for second instars, Web-Cote bands caught the largest number of second instar *L. delicatula* as well.

Currently, the most effective tool to locally reduce *L. delicatula* populations is a two-pronged approach involving the removal of most *A. altissima* trees on a property combined with systemic treatment of the few remaining *A. altissima* trees with a neonicotinoid insecticide such as dinotefuran. This creates ‘trap trees’, which are highly attractive to *L. delicatula* but also toxic when fed upon. This approach has been demonstrated to greatly reduce *L. delicatula* populations ([S. Spichiger, unpublished data](#)).

Bringing *L. delicatula* under control regionally will require the ability to delineate the spatial extent of its population, which requires sensitive detection tools. Prior to this work, detection tools were extremely limited. Although the distance over which *L. delicatula* may be attracted to these lures is unknown, high release rate methyl salicylate lures and improved traps may enhance our detection capabilities several-fold. Although they can aid in detection of low density populations, these traps and lures probably rely greatly on their fortuitous placement on trees that are already infested with *L. delicatula*, because *L. delicatula* nymphs and adults usually engage in a cyclic behavior of walking up trees, hopping or falling, and

ascending the same or nearby trees (Kim et al. 2011). However, recent observations in Pennsylvania suggest that conditions may exist when large numbers of adults will fly during a brief window of time in mid to late September. Because the distribution of *L. delicatula* throughout a landscape and tree-to-tree can be extremely patchy and attractive host odors are abundant in the environment, it will likely not be possible to develop sensitive, semiochemical detection tools in the absence of a powerful long-range attractant, such as a pheromone. Although they appear to aggregate, no evidence of a pheromone has been demonstrated for *L. delicatula*, and we are not aware of other examples of pheromones in Fulgoridae. However, our ongoing research includes trying to determine whether pheromones exist in *L. delicatula*. With there being a high risk of human-assisted movement, it is critical to develop improved detection tools quickly and to identify high-risk pathways to reduce the insect's spread. Survey and detection should be conducted in high-risk areas throughout the country so that new populations can be discovered and mitigated before they grow to an unmanageable size.

The research described here includes the first attempt in the United States to study the behavior and chemical ecology of *L. delicatula*, initiated within months of its discovery in Pennsylvania with the urgent goal of developing new detection tools. When this work commenced early in 2015, literature on the biology, chemical ecology, and trapping of this species was sparse; eggs were still overwintering, and first instars had not yet been observed by any of the coauthors. Use of electroantennographic detection coupled with gas chromatography (GC-EAD) would have greatly enhanced our ability to search for and find compounds to test for attraction, but this analysis was not immediately possible on this species and required method development. Our approach allowed us to move to field testing quickly. Exploration for additional kairomones and possible pheromones using GC-EAD is in progress.

Acknowledgments

We extend our thanks to the field crews at the Pennsylvania Department of Agriculture for collecting *L. delicatula* for laboratory studies, and for carrying out field studies in Pennsylvania. We also thank the students at Beijing Forestry University in Beijing, and Z. Longwa from Anhui Agricultural University in Hefei, for conducting field studies there. We are grateful to Bedoukian for synthesizing and providing (*E,E*)- α -farnesene for laboratory and field testing. For developing and providing lures for field testing, we thank D. Czokajlo from Alpha Scents, P. Maclean and K. Ellis from Hercon Environmental, and S. Sexton from Sinogreen. We also thank D. Czokajlo from Alpha Scents for providing sticky bands to test. We thank E. Smyers for providing grape plants for rearing and aerations; J. McKay for assistance in rearing, bioassays, and formulating in-house lures for field studies; M. Hicken for assistance in rearing; and G. Setliff for collecting insects. We thank S. Adams, M. Murray, and D. Lafoon for help gathering *A. altissima* for rearing *L. delicatula* in the laboratory. Finally, we thank all of the property owners who allowed field work to be conducted on their properties, without which we could not have done this work. This research was funded by USDA Section 10007 of the 2014 Farm Bill (2015: 6.0380.02, 6.0380.04; 2016: 3.0187.01, 3.0187.03; 2017: 6R.3.0320.01, 6R.3.0320.03, 6R.3.0320.04). Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture (USDA). USDA is an equal opportunity provider and employer.

References Cited

Albouchi, F., I. Hassen, H. Casabianca, and K. Hosni. 2013. Phytochemicals, antioxidant, antimicrobial and phytotoxic activities of *Ailanthus altissima* (Mill.) Swingle leaves. *S. Afr. J. Bot.* 87: 164–174.

- Barringer, L. E., L. R. Donovall, S-E. Spichiger, D. Lynch, and D. Henry. 2015. The first new world record of *Lycorma delicatula* (Insecta: Hemiptera: Fulgoridae). *Entomol. News.* 125: 20–23.
- Boeve, J. 1999. Chemical ecology of the European apple sawfly, *Hoplocampa testudinea*. *IOBC WPRS Bulletin.* 22: 15–19.
- Choi, D-S., D-I. Kim, S-J. Ko, B-R. Kang, J-D. Park, S-G. Kim, and K-J. Choi. 2012. Environmentally-friendly control methods and forecasting the hatching time *Lycorma delicatula* (Hemiptera: Fulgoridae) in Jeonnam Province. *Korean J. Appl. Entomol.* 51: 371–376.
- Cooperband, M. F., A. A. Cossé, T. H. Jones, D. Carrillo, K. Cleary, I. Canlas, and R. Stouthamer. 2017. Pheromones of three ambrosia beetles in the *Euwallacea fornicatus* species complex: ratios and preferences. *PeerJ.* 5: e3957.
- Cooperband, M. F., R. Mack, and S-E. Spichiger. 2018. Chipping to destroy egg masses of the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae). *J. Insect. Sci.* 18(3): 7.
- Dara, S. K., L. Barringer, and S. P. Arthurs. 2015. *Lycorma delicatula* (Hemiptera: Fulgoridae): a new invasive pest in the United States. *J. Integ. Pest Manag.* 6: 20.
- El Ayeb-Zakhama, A., S. Ben Salem, L. Sakka-Rouis, G. Flamini, H. Ben Jannet, and F. Harzallah-Skhirri. 2014. Chemical composition and phytotoxic effects of essential oils obtained from *Ailanthus altissima* (Mill.) swingle cultivated in Tunisia. *Chem. Biodivers.* 11: 1216–1227.
- Francese, J. A., J. B. Oliver, I. Fraser, D. R. Lance, N. Youssef, A. J. Sawyer, and V. C. Mastro. 2008. Influence of trap placement and design on capture of the emerald ash borer (Coleoptera: Buprestidae). *J. Econ. Entomol.* 101: 1831–1837.
- Francese, J. A., D. J. Crook, I. Fraser, D. R. Lance, A. J. Sawyer, and V. C. Mastro. 2010. Optimization of trap color for emerald ash borer (Coleoptera: Buprestidae). *J. Econ. Entomol.* 103: 1235–1241.
- Haye, T., T. Garipey, K. Hoelmer, J-P. Rossi, J-C. Streito, X. Tassus, and N. Desneux. 2015. Range expansion of the invasive brown marmorated stinkbug, *Halyomorpha halys*: an increasing threat to field, fruit and vegetable crops worldwide. *J. Pest Sci.* 88: 665–673.
- Jang, Y., H-G. An, H. Kim, and K-H. Kim. 2013. Spectral preferences of *Lycorma delicatula* (Hemiptera: Fulgoridae). *Entomol. Res.* 43: 115–122.
- Kim, J. G., E-H. Lee, Y-M. Seo, and N-Y. Kim. 2011. Cyclic behavior of *Lycorma delicatula* (Insecta: Hemiptera: Fulgoridae) on host plants. *J. Insect Behav.* 24: 423–435.
- Kim, H., M. Kim, D. H. Kwon, S. Park, Y. Lee, J. Huang, H-S. Lee, K-J. Hong, Y. Jang, and S. Lee. 2013. Molecular comparison of *Lycorma delicatula* (Hemiptera: Fulgoridae) isolates in Korea, China, and Japan. *J. Asia-Pacific Entomol.* 16: 503–506.
- Mastelic, J., and I. Jerkovic. 2002. Volatile constituents from the leaves of young an old *Ailanthus altissima* (Mill.) swingle tree. *Croatica Chem. Acta.* 75: 189–197.
- Miller, D. R., and R. J. Rabaglia. 2009. Ethanol and (-)-alpha-Pinene: attractant kairomones for bark and ambrosia beetles in the southeastern US. *J. Chem. Ecol.* 35: 435–448.
- Moon, S-R., S-R. Cho, J-W. Jeong, Y-H. Shin, J-O. Yang, K-S. Ahn, C. Yoon, and G-H. Kim. 2011. Attraction response of spot clothing wax cicada, *Lycorma delicatula* (Hemiptera: Fulgoridae) to spearmint oil. *J. Korean Soc. Appl. Biol. Chem.* 54: 558–567.
- Parra, G., H. Moylett, and R. Bulluck. 2017. Technical working group summary report - spotted lanternfly, *Lycorma delicatula* (White, 1845), pp. 42. USDA-APHIS-PPQ-CPHST, Raleigh, NC.
- PDA. 2018. Quarantine. http://www.agriculture.pa.gov/Plants_Land_Water/PlantIndustry/Entomology/spotted_lanternfly/quarantine/Pages/default.aspx (accessed 19 July 18).
- Prokopy, R. J., P. L. Phelan, S. E. Wright, A. J. Minalga, R. Barger, and T. C. Leskey. 2001. Compounds from host fruit odor attractive to adult plum curculios (Coleoptera: Curculionidae). *J. Entomol. Sci.* 36: 122–134.
- Rohlf, J. F., and R. R. Sokal. 1995. *Statistical tables.* W. H. Freeman and Company, New York.
- Snyder, A., M. Kasson, S. Salom, D. Davis, G. Griffin, and L. Kok. 2013. First report of Verticillium wilt of *Ailanthus altissima* in Virginia caused by *Verticillium nonalfalfae*. *Plant Dis.* 97: 837–837.

- Sobotník, J., R. Hanus, B. Kalinová, R. Piskorski, J. Cvacka, T. Bourguignon, and Y. Roisin. 2008. (E,E)-alpha-farnesene, an alarm pheromone of the termite *Prorethra canalifrons*. *J. Chem. Ecol.* 34: 478–486.
- Sokal, R. R., and J. F. Rohlf. 1995. *Biometry*. W. H. Freeman and Company, New York.
- Song, M. K. 2010. Damage by *Lycorma delicatula* and chemical control in vineyards, pp. 39, *Agricultural biology and applied entomology*. Chunbuk National University, Korea.
- Suckling, D. M., L. D. Stringer, B. Bunn, A. M. El-Sayed, and R. K. Vander Meer. 2010. Trail pheromone disruption of red imported fire ant. *J. Chem. Ecol.* 36: 744–750.
- Tholl, D., W. Boland, A. Hansel, F. Loreto, U. S. Röse, and J. P. Schnitzler. 2006. *Practical approaches to plant volatile analysis*. *Plant J.* 45: 540–560.
- Tomisawa, A., S. Ohmiya, H. Fukutomi, K. Hayashi, and T. Ishikawa. 2013. Biological notes on *Lycorma delicatula* (White) (Hemiptera, Fulgoridae) in Ishikawa Prefecture, Japan. *Jpn J. Entomol.* 16: 3–14.
- VDACS. 2018. New Invasive Pest Detected in Virginia. Virginia Department of Agriculture and Consumer Services, <http://www.vdacs.virginia.gov/press-releases-180208-spottedlanternfly.shtml> (accessed 19 July 18).
- Wei, J., and L. Kang. 2011. Roles of (Z)-3-hexenol in plant-insect interactions. *Plant Signal. Behav.* 6: 369–371.
- Yoon, C., S-R. Moon, J-W. Jeong, Y-H. Shin, S-R. Cho, K-S. Ahn, J-O. Yang, and G-H. Kim. 2011. Repellency of lavender oil and linalool against spot clothing wax cicada, *Lycorma delicatula* (Hemiptera: Fulgoridae) and their electrophysiological responses. *J. Asia-Pacific Entomol.* 14: 411–416.