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Evidence of Receptivity to Vibroacoustic Stimuli in the Spotted Lanternfly *Lycorma delicatula* (Hemiptera: Fulgoridae)

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Abstract

The spotted lanternfly *Lycorma delicatula* White (Hemiptera: Fulgoridae) is a polyphagous insect pest that invaded the United States in 2014, in Berks County, Pennsylvania. It has since spread to several northeastern states and poses a significant threat to northeastern grape production. Most studied species of Hemiptera are known to communicate intraspecifically using some form of substrate-borne vibrational signals, although such behavior has not yet been reported in *L. delicatula*. This report demonstrates that adult and fourth-instar *L. delicatula* were attracted towards broadcasts of 60-Hz vibroacoustic stimuli directed to a laboratory arena and test substrate, which suggests that both adults and fourth instar nymphs can perceive and respond to vibrational stimuli.

Key words: spotted lanternfly, acoustic trapping, sound, vibration, taxis

The spotted lanternfly (SLF) *Lycorma delicatula* White (Hemiptera: Fulgoridae) is a polyphagous, phloem-feeding invasive fulgorid planthopper native to China. It was found in Pennsylvania in 2014 and has now spread to several neighboring states (Pennsylvania Department of Agriculture 2018, New York IPM 2019). Although SLF feeds on over 100 plant species (Dara et al. 2015), the preferred host of this economic pest is tree-of-heaven, *Ailanthus altissima* (Mill.) Swingle (Sapindales: Simaroubaceae), also invasive from China and broadly distributed throughout the United States (Derstine et al. 2020). SLF can seriously damage or even kill grapevines (*Vitis* spp.), and they threaten a range of other agricultural commodities such as fruit trees, nursery stock, and timber (Baker et al. 2019).

Vibroacoustic communication, in the form of substrate-borne vibrations on the host plant (Roberts and Wickings 2022), has been observed in many hemipteran species (Bedoya et al. 2020, Cocroft et al. 2005, Soulier-Perkins et al. 2007, Zych et al. 2012). This potential mode of communication in SLF remains unstudied, but anecdotal reports indicate adults exhibit positive phonotaxis toward the 60 Hz buzz produced by electrical wires, which can induce vibrations in nearby substrates (Mankin et al. 2018). If vibroacoustic stimuli are attractive to SLF, then this behavior might be manipulated for purposes of developing traps with bimodal (pheromonal + vibrational) stimuli (Polajnar et al. 2019a,b; Zapponi et al. 2022) or for mating disruption, as demonstrated in other hemipterans (Lujo et al. 2016, Andrade et al. 2020, Zapponi et al. 2022). Here, we demonstrate that SLF fourth instar nymphs and adults are attracted to a 60 Hz stimulus under experimental conditions.

Materials and Methods

Specimen Collection

Unlike Asian citrus psyllids, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) (Paris et al. 2013) and other pest species, laboratory rearing protocols have not yet been developed for SLF. Instead, the specimens used in this study were shipped from their current range to a biosecure laboratory for experimental sound playback experiments. Fourth instar nymphs and adult males and females were collected from a research site in Northampton County, northeastern Pennsylvania from 20 July to 17 August 2020. Field collected specimens were shipped weekly, as described previously (Derstine et al. 2020), to the Otis Laboratory Insect Containment Facility in Buzzards Bay, MA according to the conditions set in permits from Pennsylvania Department of Agriculture (PDA) (PP3-0123-2015) and U.S. Department of Agriculture (USDA) (P526P-15-00152 and P526P-17-04376). The live insects were then maintained at 23°C on potted *A. altissima* plants in insect cages (47.5 × 47.5 × 93.0 cm L × W × H, BugDorm-4S4590, Megaview Science Co. Ltd., Taiwan) with natural daylight in a greenhouse within the biosecure facility. Fourth instar nymphs and adults were tested in bioassays therein.

Bioassay Setup

In a quiet room in the containment facility, a paper-floored arena with a printed circle (20 cm in diameter) was placed on a plywood platform and enclosed in a tulle-covered, 48 by 41 by 30 cm (Length by Width by Height) acrylic frame. This arena was surrounded with white paper to screen out visual cues. A 6.5 by 3.5 cm window was cut into the top of one side of the paper arena enclosure for the observer to view from one hidden position. A speaker (FBA_F10-GY, MIFA Innovations, Newark, DE) was placed on the platform outside of the paper screen, pointing across the center of the circle from either a NE, SW, SE, or NW position to systematically accommodate for differences in lighting or surface that might influence insect movement in the arena. During 'Speaker On' operation, a continuous 60 Hz sine wave produced by a tone generator (Sonic, Von Bruno, Seattle, WA) was streamed from a cell phone to the speaker using Bluetooth (Bluetooth SIG, Inc., Kirkland, WA). The tone playback was 55.7 dB as measured with a sound meter (Extech Instruments Corp., 407727 Digital Sound Level Meter, Waltham, MA). The vibrations exciting the arena substrate by the speaker tone were measured with a laser vibrometer (VibroGo VGO-200, Polytec, Inc., Irvine, CA) at the same position where insects were released into the arena. The measured vibration level was 60 ± 5 Hz and 55.7 vibration decibels (VdB) relative to the 0 dB reference of 10^{-9} m/s, which was approximately 5.7 dB above the background vibration level with 'Speaker Off'. For each replicate, while the stimulus was on, a vial containing one previously untested insect was placed at the center of the circular arena, and after 30 s the vial was removed, allowing the insect to move freely. The insect was observed for two minutes or until it reached the edge of the arena. The direction of exit from the circle was recorded in degrees from speaker axis (0–360°). The paper floor was replaced after each insect replicate. Replicates also were obtained from nymphs and adults released at the center of the arena when the speaker was turned off. There were 16 replicates each of nymphs exposed to active speakers, and 20 replicates each of adults (alternating males and females) exposed to active speakers, nymphs exposed to inactive speakers, and adults exposed to inactive speakers.

Statistical Analysis

Circular data, such as locomotor activity measured in degrees or radians from a centroid in a circular arena, are fundamentally different from linear data due to its periodicity and directionality (Cremers and Klugkist 2018). As such, our data were analyzed using the V-test for nonuniformity in relation to a known mean direction, a variant of the Rayleigh distribution (Durand and Greenwood 1958) that allows the mean of the alternative distribution to be specified prior to application of the test (Landler et al. 2018). The data were analyzed in MATLAB R2021b (The MathWorks Inc., Natick, MA), and the Matlab Circular Statistics Toolbox (Berens 2009) V-test was applied to determine the

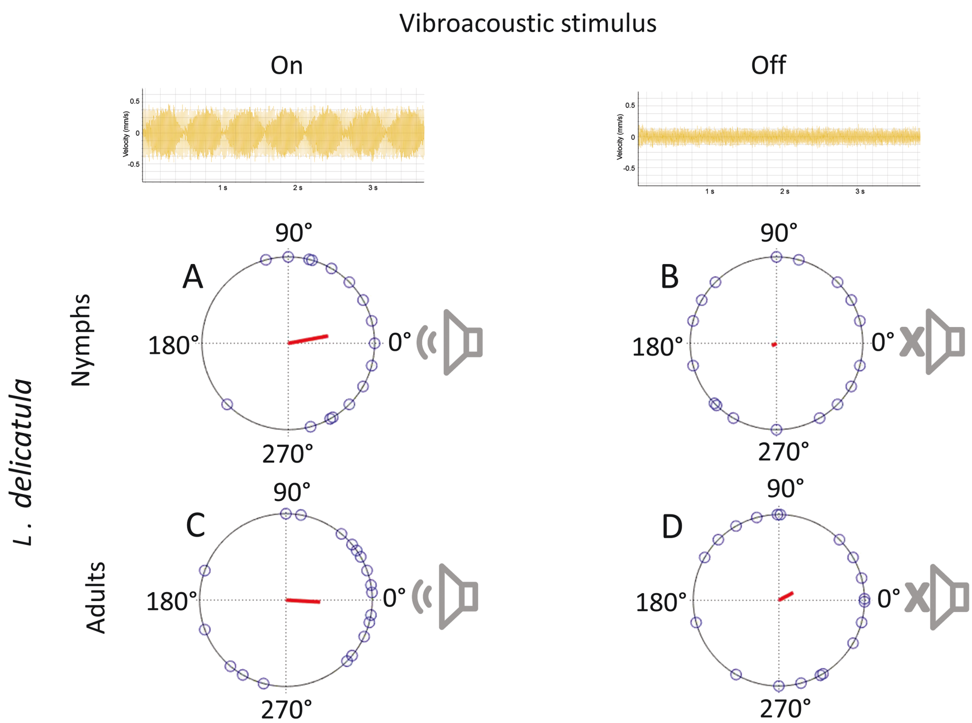

Fig. 1. *Lycorma delicatula* nymphal and adult behaviors under exposure to 60 Hz vibroacoustic stimulus (On or Off). Vibrometer recordings at the release position are shown above. The directions where the insects exited the 20-cm diameter circle after being released at its center are indicated by open circles on the 0–360° axis, which is a normalized circle of radius = 1. Solid lines pointing from the center of each circle indicate the mean direction and its magnitude for each stimulus condition. The magnitude of the mean direction has a maximum value of 1, obtained if all insects exited the test circle at the same angle.

statistical significance of the null hypothesis that the mean direction of exit from the bioassay circle was different from the speaker direction.

Results

Vibrations measured at the release position of the arena when the stimulus tone was on and off are shown in Fig. 1. Nymphs and adults exited the bioassay circle in directions that clustered towards the speaker axis at 0 radians (0°) when it was in operation but spread more uniformly when the speaker was silent (Fig. 1). The 16 fourth instar nymphs moved in a mean direction of 0.18 radians (10.4°) from the 0-radian direction of the operating speaker (Fig. 1A). The normalized length of the direction vector for fourth instar nymphs was 0.47, in comparison to a normalized length of 1 that would occur if all nymphs moved in the same direction. Application of the V-test indicated that the effect of the operating speaker on nymph movement was statistically significant (df = 15, V-test $P = 0.004$, Standard Deviation [SD] = 1.02 radians). When the speaker was silent (Fig. 1B), the fourth instar nymphs had a mean direction of −2.63 radians (−151.03°) and the normalized length of the direction vector was only 0.06 and not significantly different from 0 (df = 19, $P = 0.630$), indicating that the individual directions were uniformly distributed (Berens 2009).

The 20 adult SLF moved in a mean direction of −0.057 radians (−3.2°) (Fig. 1C) and had a normalized length of 0.3978. Application of the V-test to the adult movement directions indicated that the effect of the operating speaker on the mean adult direction was statistically significant (df = 19, $P = 0.006$, SD = 1.36 radians). When the speaker was inactive, however, the mean direction was 0.492 radians (28.2°) and had a normalized length of 0.1891 (df = 19, $P = 0.146$), which was not significantly different from 0 (Fig. 1D).

Discussion

Our findings show that both fourth instar and adult SLF walk preferentially towards a 60 Hz vibroacoustic stimulus. Such findings suggest further investigation of the social behavior of this invasive species, and the potential role of sound and/or substrate-borne vibrational signals in its intraspecific interactions. SLF adults spend most of their time on woody surfaces of trees as they feed, walk, and aggregate (Cooperband and Murman 2022), and the aposematic colorings of fourth instar and adult SLF suggest that they may benefit defensively from aggregation behaviors (Ruxton and Sherratt 2006). The mechanisms used by SLF to form aggregations are not fully understood, but may also involve semiochemicals from their honeydew secretions (Faal et al. 2022). The 60 Hz vibrations are within the lower range of signals produced by other hemipterans during mating communication (Čokl and Virant-Doberlet 2003, Čokl 2008). Field studies that monitor vibrational signals in host trees concurrently with monitoring of SLF adult and nymph behavior during periods when SLF are beginning to aggregate and/or during periods of mating may lead to insights that could be applied for vibrational trapping (Caorsi et al. 2021), pheromonal–vibrational trapping (Polajnar et al. 2019a,b, Zapponi et al. 2022), interference with aggregation behavior (Zych et al. 2012, Bedoya et al. 2020), or other pest management activities.

Our experiments did not identify whether insects were responding to airborne pressure waves, particle motion, or substrate-borne cues transduced from the 60 Hz tone to the arena substrate, but fourth instars and adult insects typically have mechanoreceptors on their bodies that sense substrate vibrations (Tuthill and Wilson 2016). Although several studies describe sensory receptors for SLF, airborne pressure receptors (tympana) have not been described (Hao et al. 2016, Wang et al. 2018). Previous studies already have demonstrated that airborne sound can elicit vibrations in plants that influence hemipteran insect behaviors (Saxena and Kumar 1980). In the absence of additional information, it is thus likely that all or much of the playback signal perceived by SLF is substrate-borne and is detected by mechanoreceptors.

Substrate-borne vibrational communication is ubiquitous in numerous groups of insects (Virant-Doberlet and Cokl 2004, Cocroft and Rodriguez 2005), and prevalent in many hemipteran pests of agricultural crops (Cokl et al. 2005, Blassioli-Moraes et al. 2014; Polajnar et al. 2015, 2016). Often these signals are transmitted across the stems and leaves of the host plant, as in the potato leafhopper, *Empoasca fabae* Harris (Hemiptera: Cicadellidae) on grape, and are associated with localizing a mate, assessing mate quality, as well as with courtship and pair-formation (Cokl and Virant-Doberlet 2003), and communication within aggregations containing adults (Mankin et al. 2020) or adults and nymphs (Zych et al. 2012).

In addition to the interest in vibrational trapping or in co-opting of signals used by pest insects to form or interact in aggregations, as noted above, there has been continued interest in mating disruption, either through disruption of chemical communication (Silverstein 1981, Plettner 2002) or through development of disruptive tools for insect management that make use of substrate-borne and/or airborne acoustic signals (Eriksson et al. 2012, Polajnar et al. 2015) or combinations of air- and substrate-borne stimuli (Gordon et al. 2019; Polajnar et al. 2019a,b). Substrate-borne vibrations often are important components of pre- and/or post-copulatory behavior and reproductive isolation in invertebrates (Eberhard 1991, Hill 2015). Recent work on leafhopper species infesting Italian and California vineyards (Nieri et al. 2017) has shown that nonfocal males produce a vibrational 'jamming' signal to disrupt the choreographed vibrational courtship duet of a rival male and female (Mazzoni et al. 2009a), and researchers have shown that experimental playbacks of these substrate-borne vibrational noises can likewise interfere with mating and reproduction of the species in both laboratory and field settings (Mazzoni et al. 2009b). Current work is underway in large wine vineyards in northern Italy (Polajnar et al. 2016) and for table grape vineyards in central California (Mazzoni et al. 2017, Krugner and Gordon 2018) to examine the scalability of this approach for cicadellid leafhoppers such as the glassy-winged sharpshooter, *Homalodisca vitripennis* Germar (Hemiptera: Cicadellidae) and the Nearctic leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae). The present work indicates that such methods may be applicable to controlling SLF, especially given the attraction of both adults and nymphs to 60 Hz signals.

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