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Short communication

Orientation of flight for physically disturbed spotted lanternflies, *Lycorma delicatula*, (Hemiptera, Fulgoridae)

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ABSTRACT

The spotted lanternfly, *Lycorma delicatula*, (Hemiptera, Fulgoridae) is an invasive pest to Korea and the United States, originating from China or Southeast Asia. Immature *L. delicatula* feed on a wide range of plants, but the adults are more host-selective, often preferring the tree of heaven, *Ailanthus altissima*. We performed field studies to evaluate adult movement in relation to *A. altissima* after disturbance. The *Ailanthus trees* were in a mixed suburban forested situated at the southern border of an open grassy field. Female adult lanternflies were manually disturbed from feeding on the trunks of large *A. altissima* trees. In the first experiment, insects were disturbed directly from the tree using a ballpoint pen to simulate a predatory attack. These insects usually flew initially southward away from the tree line toward a sunlit field, but turned northward back toward the tree line. In the second experiment, to simulate an initially successful predatory attack, they were manually taken from trees, and allowed to escape. The females immediately opened their wings in an apparent aposematic display. They then either immediately flew toward the sunlit open field, or remained with their wings splayed open for a prolonged period.

Introduction

The spotted lanternfly, *Lycorma delicatula,* is an invasive insect that has been recently introduced to North America, likely from indigenous populations in China. The first record of this species in the United States occurred in 2014 in Berks County, Pennsylvania (Barringer et al., 2015). It had also been previously introduced from China to Korea (Kim and Kim, 2005; Han et al., 2008) and Japan (Kim et al., 2013; Tomisawa et al., 2013). The species has now spread to several counties in eastern Pennsylvania (Parra et al., 2017), and there is concern of its certain continued spread over a broader geographic area in North America if it cannot be controlled or eradicated (Dara et al., 2015).

L. delicatula is known to be strongly associated with its primary host, the tree of heaven, *Ailanthus altissima* (Sanyang, 1992; Lee et al., 2009), both in its native and introduced populations. Wild and cultivated grapevines, *Vitis*, can harbor all life stages of the insect. Younger nymphs have a broader host range, but do not develop to adulthood as well on alternative hosts (Lee et al., 2009). Adults can be found feeding on many parts of *Ailanthus altissima* trees. Often within the span of a day or two, *L. delicatula* will ascend to the canopy of the tree and then drop, before ascending the tree again (Kim et al., 2011). Observation of this behavior has led to the development of placing sticky bands on tree trunks as a method of trapping *L. delicatula* (Choi et al., 2002).

Much still remains unknown about the life history of *L. delicatula*, including an understanding of flight as it relates to its dispersal, mating behavior, and predator avoidance. It has been determined that there are spectral preferences in the orientation toward light in laboratory assays, with blue and ultraviolet frequencies causing the greatest attraction (Jang et al., 2013). No such stimuli have yet been utilized in trapping technology. Semiochemicals might also be related to flight behavior and exploitable for control.

Another identifiable behavior of *L. delicatula* is its tendency to jump and display the aposematic red coloration of its hind wings as an adult when it is disturbed (Kang et al., 2011, 2017). The insect is likely to be highly unpalatable because it accumulates high levels of alkaloids (Xue and Yuan, 1996). It was determined that females are more likely than males to jump and fly when disturbed. Much still remains unknown about these behaviors, such as how effective they may be for deterring predation (Kang et al., 2011), and whether visual behavioral displays are coordinated with other environmental cues.

In this study two experiments were performed to better understand the aposematic display and escape response of disturbed *L. delicatula*, and whether such behaviors are affected by the location of nearby host plants. These experiments included observing both the disturbance of insects from the *A. altissima* trees they were resting upon, and determining their direction of flight after being captured and released. We

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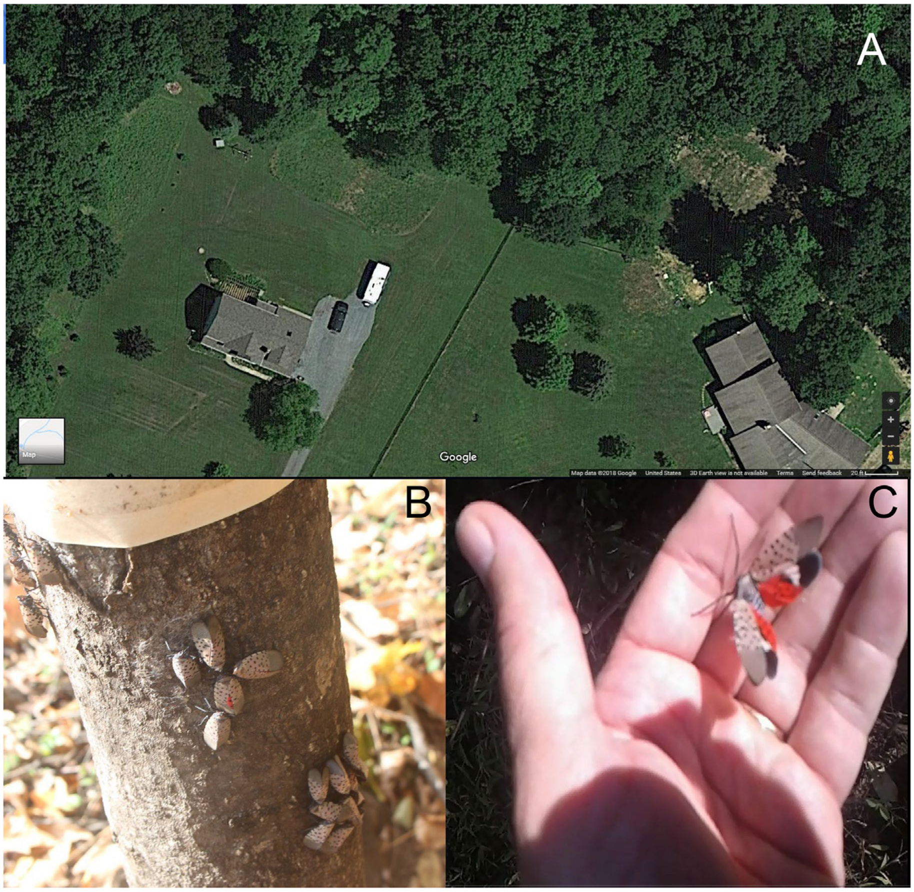

Fig. 1. Experiments were performed on a property in Boyertown, PA along a tree line of a forest bordering a residential lawn (A). Flight was induced by disturbing *L. delicatula* from the base of large *A. altissima* trees (A) with a ballpoint pen or manual capture and release (B).

were interested in characterizing wing displays and whether there was any evidence that disturbed insects might fly in a characteristic pattern toward or away from host plants populated with aggregations of *L. delicatula*.

Methods

For both experiments, insects were selected from large all-female aggregations on several large *A. altissima* trees (> 15 cm diameter). Such female aggregations (> 99%) have not been previously described in the literature, but were observed at three sites in the area in 2015. There were thus not enough males available to include in replicated experiments. The experimental plot was at the edge of a forest adjacent to a large residential lawn south of the tree line. The tree line was an approximately 30° clockwise rotation from a precise east-west orientation (Fig. 1A). All experiments were conducted midday in August and early September, and thus the sun was always shining toward the trees from the field. In this environment, ambient light was always greater in the open field versus inside the forest. If a northward facing forest edge had been used, there would have been micro-environments of very high light intensity within the forest.

Disturbance experiment 1

A reference grid was established by placing twine at 50 cm intervals in lines perpendicular and parallel to the tree line. Seven lines were placed in both dimensions. The experimenter disturbed a single feeding female from the aggregation (Fig. 1B) using a ballpoint pen, while standing on the opposite side of the tree. This action was performed 34 times, with half of the disturbed insects feeding on the shaded side of the tree, and the other half on the sunlit side. All insects disturbed were 10 to 30 cm above the ground.

The grid was used as a reference to more precisely measure positions of the insect along its flight path. It was noted that many of the insects flew away from the tree line and then turned back toward the trees before resting. Thus up to three points were marked on the ground within the grid, so that more precise measurements could be approximated from the visual observation: 1) The starting position, or location of the tree from which it was disturbed 2) the point of any direction change, and 3) the final resting place. A marker was placed at the change of direction point and the point where the insect landed, and a tape measure was used to determine the vector position of each point to the nearest cm. This allowed computation of flight distances and angle with respect to the tree line at each step in the flight trajectory.

Disturbance experiment 2

This experiment involved first manually removing *L. delicatula* females from aggregations on similar large *A. altissima* trees, holding them each gently in a closed hand. The experimenter then stood 2 m from the tree line with hand outstretched westerly 150 cm above the ground, parallel to the tree line and in direct sunlight so that there were no shadows enveloping the hand. After five seconds the hand was slowly opened, palm faced upward (Fig. 1C). This procedure resulted in the insects being oriented head-first toward the tree line when released. The subsequent behaviors of *L. delicatula* were observed including the duration of time they remained on the hand, whether the wings were ever opened, if flight behavior ensued, and the direction of any flight (toward tree line or into sunlit field).

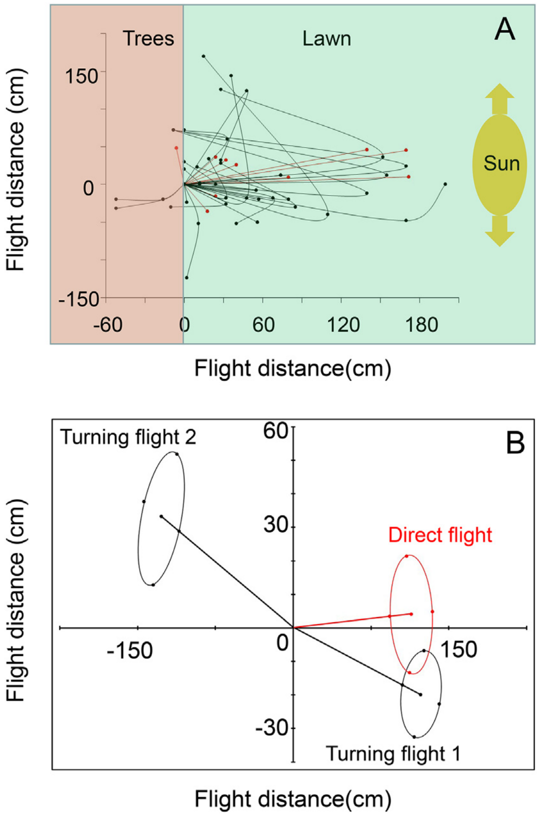

Fig. 2. All flight trajectories of *L. delicatula* females after disturbance from trees along a forest edge (A) and an estimate of the mean angle and distance travelled (B) for lanternflies that either did not turn (red) or those that made a pronounced inflight turn (black). For those turning, the angles before and after turning are shown. Ellipses are centered on the mean direction and distance of the flight and are extended to one SEM of both parameters. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Statistical analysis

The distance and angles of each disturbed insect were calculated using their beginning and ending coordinates on the grid. Circular statistics were calculated using NCSS™ 12 software, and all other statistical analyses were performed using SAS™ 9.4 software. When it was visually assessed that there was a pronounced turn (> 90°), the flight was treated as having two separate portions, which were analyzed separately. The flights were also analyzed by grouping them according to whether the initial position was on the shaded or sunny side of a tree. Tables 1 and 2 describe the statistical parameters estimated, and the tests performed. Bonferroni corrections were always made for any test repeatedly applied.

Results

In the first disturbance experiment, nearly all of the insects (91%) made flights toward the sunlit field. The initial flight was immediately followed by a turn in 59% of cases; usually back toward the tree line. The initial mean flight direction and its distribution did not differ regardless of whether there was a subsequent return flight, nor depending on which side of the tree they were on when disturbed (Tables 1 and 2). The concentration of the distributions usually did not differ between any of the flight comparisons Table 2. There was a higher concentration in the distribution of directions for initial flights originating from the sunny side versus the shaded side, perhaps indicating that such insects were better able to orient toward sunlight. However, there should be caution because there was a significant departure from the assumption of a von Misis distribution in this case. There were no differences between the various types of flight trajectories with respect to the length of the flights observed (Tables 1 and 2).

Recording the escape response of hand-released *L. delicatula* was repeated with 50 different females (Fig. 1B). In every case the females immediately opened their wings to display their bright red coloration. The females followed this display with discretely different behaviors. The first type consisted of almost immediately (< 5 s) flying toward the sun, which required a 180° turn (Table 3). Alternatively, they often stayed on the hand longer and gradually closed their wings. From this latter group that closed their wings and stayed longer, differences in the subsequent flight behavior was observed. Unlike the insects that quickly flew into the sun without closing their wings, those that had closed their wings were more likely to either fly back toward the tree line or to remain on the hand for > 2 min without flying.

Discussion

There are many potential mechanisms by which predation is avoided during flight that may including for example evasive maneuvering (Robertson and Reye, 1992; Jantzen and Eisner, 2008). For *L. delicatula* females, the flight observed in response to disturbance seemed to involve the display of their red coloration as they flew toward open sunlit areas. There were also no signs of horizontal casting in the flight paths, which would be characteristic of the optimotor anemonotaxis involved in odor tracking. This action of entering areas where they will be highly visible may have evolved to amplify the effect of their aposematic coloration to more effectively advertise their distastefulness (Kang et al., 2011, 2017). Kang et al. (2017) documented that such displays were particularly more common early in the adult period, which corresponds to the age of females used in this study. They also noted a deimatic display for predator avoidance, which was influenced by factors such as the severity of the disturbance, as well as sex and seasonal progression (Table 3).

We did not observe any such deimatic displays, but we used only females that were actively feeding in the early adult period, and provided a lower intensity disturbance. Thus our study provides new

Table 1
Summary statistics for flight distance and orientation of *L. delicatula* females disturbed from trees adjacent to an open field (Fig. 2). Direct fliers did not turn (DF), while the trajectories of turning fliers had two distinct portions (TF1 & TF2) marked by angular difference > 90°. Initial flights (DF & TF1) were later pooled and grouped according to whether they initiated on the shaded (SHD1) or sunny side of trees (SUN2). Return flights (TF2) were respectively grouped as SHD2 or SUN2.

Group	N	Distance(cm) ± SEM	Von Misis concentration	Direction (°) ± SEM	Hypothesized direction (°)	Watson & Williams test
DF	14	76.0 ± 13.7	1.6	3.1 ± 13.3	0	0.0455 (n.s.)
TF1	20	84.4 ± 12.2	2.2	−13.7 ± 9.0	0	1.4253 (n.s.)
TF2	20	90.8 ± 14.2	1.5	158.6 ± 13.3	180	1.8549 (n.s.)
SHD1	17	74.0 ± 13.7	1.3	1.9 ± 17.1	0	0.0104 (n.s.)
SUN1	17	87.8 ± 13.8	5.4	−12.1 ± 6.2*	0	3.2203 (n.s.)
SHD2	10	60.8 ± 11.9	1.1	177.2 ± 24.1	180	0.0126 (n.s.)
SUN2	10	120.8 ± 21.4	1.8	144.2 ± 12.0	180	3.7162 (n.s.)

* This distribution does not match the assumption of a von Misis distribution (Cox's test statistic 13.2, P < .05).

Table 2
Summary statistics of statistical comparisons of flight distance and orientation between groupings of trajectories of *L. delicatula* females disturbed from trees adjacent to an open field as depicted and described in detail in Fig. 2 and Table 1.

Comparison	Flight distance (student's *t*)	Flight angle properties		
		Concentration: homogeneity	Distribution: uniform scores	Direction: Watson & Williams
DF vs. TF1	−0.45 (32 d.f., n.s.)	0.0244 (n.s.)	5.22 (n.s.)	0.993 (n.s.)
TF1 vs TF12	−0.59 (19 d.f., n.s.)[a]	0.936 (n.s.)	25.8[†]	206[†]
SHD1 vs. SUN1	−0.76 (32 d.f., n.s.)	8.513[*]	3.95 (n.s.)	0.667 (n.s.)
SHD2 vs. SUN2	−2.35 (18 d.f., n.s.)	0.343 (n.s.)	1.20 (n.s.)	1.43 (n.s.)

[a] Paired *t*-test, all others are two-sample, equal variance.
[*] $p < .05$.
[†] $p < .001$.

Table 3
Fifty *L. delicatula* females were captured and manually released. All displayed their red hind wings, but they either immediately flew away or slowly closed their wings at distinctly different time scales. The percentages within each of these classes that flew toward the trees, flew toward the adjacent sunlit field, or did not fly are provided.

Initial behavior (%)	Time (s) (mean ± SEM)	Flight direction (%)[a]	Flight initiation time (s) after wing closure (mean ± SEM)
Flight (38)	2.1 ± 0.2	Sun (100)	NA
Wing closure (62)	10.8 ± 1.0	Sun (19.4)	14.0 ± 3.4
		Trees (48.4)	60.9 ± 9.9
		No flight (32.3)	NA (all > 2 mi.)

[a] The percentages flying to the sun between the groups differ ($p < .001$, Fisher's exact test).

information regarding orientation in the landscape during what Kang et al. described as “jumping” startle displays. However, it should be cautioned that it is not known to what degree all or a portion of these behaviors are specific to disturbance and predation.

In the first experiment, after the initial flight toward the open field, *L. delicatula* often flew back toward the shade and their host trees. Likewise, in the second experiment, if they eventually became acclimated to the outstretched hand after displaying their wings, they were more likely to fly toward the trees. It is likely that they need to return to the trees to feed, and also may have a greater-long-term risk of predation in the open field. The possibility for dispersal from such disturbance events appears to be limited, because our females tended to fly about a meter from the point of disturbance and then turn back toward the trees.

We can infer that a general preference for greater intensity of ambient light is most likely the cue used for such movements. It has been previously reported in laboratory choice experiments that *L. delicatula* prefer to move toward shorter UV and blue wavelengths of light (Jang et al., 2013). The greater amounts of these wavelengths of light detectable within the sunlit field versus the shaded forest, may be the cue used to direct such flight.

There may also be some implications for control. Among the strategies employed by the Pennsylvania Department of Agriculture for managing the pest have been use of sticky bands and rollers to kill as many insects as possible on heavily infested trees. There has also been the removal of preferred *A. altissima* trees, coupled with insecticide treatment of remaining trees (Parra et al., 2017). This study suggests that there is not likely to be any risk of increasing long-range dispersal from such activities, nor of encouraging movement deeper into wooded areas.

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Conflict of interest statement

Orientation of flight for physically disturbed spotted lanternflies *Lycorma delicatula*, (Hemiptera, Fulgoridae) by Michael J. Domingue*, and Thomas C. Baker.

Neither Dr. Domingue nor Dr. Baker have any relationships with people or organizations that could have biased the work or create any conflict of interest.

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