

Footprints and Ootheca of *Lycorma delicatula* Influence Host-Searching and -Acceptance of the Egg-Parasitoid *Anastatus orientalis*

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Abstract

The spotted lanternfly, *Lycorma delicatula* White (1845) (Hemiptera: Fulgoridae), is an invasive insect that was first reported in North America in Berks County, Pennsylvania, in 2014. It is a polyphagous phloem feeder that attacks over 70 plant species, threatening the agricultural, lumber, and ornamental industries of North America. Infestations of the pest have been reported in several U.S. counties, and a lack of endemic predators and parasitoids feeding on *L. delicatula* suggests a release from natural enemies in the invaded range. An egg-parasitoid *Anastatus orientalis* (Hymenoptera: Eupelmidae) was reported attacking *L. delicatula* at high rates in its native range and may play a key role in reducing its populations there. To better understand the foraging behavior of *A. orientalis*, a series of behavioral experiments were conducted to determine successful parasitism and behavioral responses to traces left by adult *L. delicatula* and to the oothecae which cover their eggs. Our results suggest that wasps detected chemical traces left by *L. delicatula* adults while walking on surfaces and exhibited a strong arrestment response. Moreover, wasps preferred to oviposit in egg masses with intact oothecae. The implications of these findings are herein discussed with regard to the exploitation of host kairomones by foraging wasps, as well as to its ability to overcome host structural defenses.

Key words: invasive species, host–parasitoid interaction, Fulgoridae, foraging behavior, spotted lanternfly

The spotted lanternfly, *Lycorma delicatula*, is a new invasive insect in North America that is native to China, Taiwan, and Vietnam, and is also invasive in Japan and Korea (Liu *et al.* 2017). Detections of this species in South Korea in 2004 followed by subsequent observations of large populations sparked increased interest in its biology, ecology, taxonomy, pest status, and control (Kim *et al.* 2013). It was first detected in North America in Berks County, Pennsylvania, in 2014, and as of October 2018, it has also been reported in Delaware, New York, Virginia, New Jersey, Maryland, Connecticut, and Massachusetts (Leach *et al.* 2019). The European and Mediterranean Plant Protection Organization categorized it in 2016 as an A1 pest (quarantine pests absent from the EPPO region), in anticipation of its potential arrival in Europe (EPPO 2019).

Lycorma delicatula is a polyphagous phloem feeder whose recorded host range includes more than 70 plant species and vines in 25 different families, such as grape, apple, birch, hops, cherry, maple,

poplar, and stone fruits (Kim *et al.* 2011, Dara *et al.* 2015). It has a univoltine life cycle in China, and in invaded regions, it overwinters as eggs laid in masses consisting of several rows that are covered in a foamy deposit to form an ootheca. Egg masses are deposited on the smooth surfaces of living and dead trees, rocks, and man-made structures (Fig. 1). Nymphs emerge in spring and early summer and pass through four developmental instars before reaching the adult winged stage in July and August (Han 2008). All life stages feed via a piercing-sucking mouthpart on a wide range of host plants, which narrows as the insect matures, with adults exhibiting a strong attraction to the tree of heaven *Ailanthus altissima* just before egg-laying in October (Kim *et al.* 2011). This attraction to *A. altissima* can be used to increase the chances of detecting *L. delicatula* in the early stages of the invasion, and key volatiles of this host plant can lead to the development of lures and behaviorally compatible traps (Lee *et al.* 2019).



Fig. 1. Dozens of *Lycorma delicatula* egg masses laid on the underside of rocks on a stone wall (a) and on a dead standing tree (b). (Image by Anna Dougherty/USDA-ARS).

Lycorma delicatula does not inflict direct damage on fruits, but feeds instead on branches and stems, causing them to wilt and lose vigor, which may result in plant death. Puncture sites result in wounds that weep sap along the bark and constitute entry points for pathogens. The insect secretes large amounts of honeydew, which together with the oozing wounds promote the growth of sooty mold that can coat leaf surfaces and fruits, thus interfering with photosynthesis and reducing plant growth, crop yield, and fruit quality (Han *et al.* 2008). Significant economic impacts have been recorded in grapevine orchards in South Korea, and mold-contaminated table grapes are deemed unmarketable (Lee *et al.* 2009). Abundant *L. delicatula* populations in South Korea also cause public annoyance as the insects intrude into people's homes and neighboring orchards in autumn (Han *et al.* 2008); this has been seen in the Pennsylvania infestation as well (Spears and Mull 2019). Potentially massive risks are posed by this invasive insect in North America; for example, Pennsylvania alone has \$18 billion worth of agricultural commodities, such as apples, grapes, and hardwoods, that are threatened by future outbreaks (Pennsylvania Department of Agriculture 2018).

Nonchemical control of *L. delicatula* includes tree banding with sticky traps, to catch nymphs and adults walking up the trees to reach the new, softer tissue. However, this method catches indiscriminately, with preliminary trials reporting impacts on nontarget organisms such as birds, reptiles, and other arthropods (T. Leskey, personal communication). Another management method is to remove the insect's preferred host plant *A. altissima*, through the application of herbicides (Pennsylvania Department of Agriculture 2018). Several contact insecticides such as deltamethrin and fenitrothion have been shown to have quick and high lethality against *L. delicatula* (Park *et al.* 2009). However, nearby populations can reinfest treated areas; thus, the use of systemic insecticides, such as dinotefuran and imidacloprid, was proposed (Kim *et al.* 2011). Leach *et al.* (2019) found that chlorpyrifos provided 100% mortality of *L. delicatula* overwintering egg masses, whereas very low efficacy was obtained from OMRI-listed compounds, indicating anticipated difficulties among organic producers in controlling this pest.

In North America, the impact of natural enemies on *L. delicatula* populations remains ambiguous. Predation on this species is scarce, possibly due to the adult camouflaged appearance of the forewings (Fig. 2) or due to natural chemical defenses acquired by feeding on *A. altissima* and sequestering cytotoxins (Kang *et al.* 2011). Moreover, sequential behavioral defenses were recorded in *L. delicatula* in response to predator attack, such as rapid escape jumping,



Fig. 2. A cryptic *Lycorma delicatula* adult in a resting state with forewings providing camouflage that serve in predator avoidance. (Image by Anna Dougherty/USDA-ARS).

deimatic display that reveals its contrasting hindwings and black/yellow banded abdomen as well as death-feigning (Kang *et al.* 2016). Two fungal entomopathogens *Beauveria bassiana* and *Batkoa major* have caused a coepizootic, which lead to localized collapse of a *L. delicatula* population in eastern United States (Clifton *et al.* 2019) and are now being evaluated for use as biocontrol agents. Also, field surveys confirmed that *Ooencyrtus kuvanae* (Hymenoptera: Encyrtidae) parasitized eggs of *L. delicatula* in Pennsylvania (Liu and Mottern 2017). In China and South Korea, native nymphal- and egg-parasitic wasps, such as *Dryinus* sp. nr. *browni* Ashmead (Hymenoptera: Dryinidae) (Yang 1994) and *Anastatus orientalis*, respectively, appear to be important in biological control. The latter is currently thought to be the major parasitoid species of *L. delicatula* in its native range, and it provides varying degrees of successful parasitization ranging from 30 to 80% depending on the region sampled and has a female biased sex ratio (Choi *et al.* 2014, Yang *et al.* 2015, Lee *et al.* 2019). *Anastatus orientalis* overwinters as mature larvae inside host eggs, with two eclosion periods reported in China, one in April–May and the second in August–September (Hou 2013).

Female parasitoid wasps often rely on chemical cues to locate their hosts in multitrophic contexts and may exploit plant synomones and host volatiles for long-range location of infested patches (Hilker and Fatouros 2015). The detection of their target life stage also depends on their ability to detect host pheromones, allomones, and kairomones (Vet and Dicke 1992, Fatouros *et al.* 2008). The first report of a parasitoid wasp using chemical traces to find its host was that of *Trichogramma evanescens* that exhibited an arrestment behavior when exposed to chemical trails left by the grain moth *Sitotroga cerealella* (Laing 1937). Rostás and Wölfling (2009) found

that female *Cotesia marginiventris* perform characteristic antennation when in contact with cuticular hydrocarbons from their larval host *Spodoptera frugiperda* footprints. Extensive studies on scelionid parasitic wasps and their heteropteran hosts have also increased our knowledge on the finely tuned exploitation of direct- and indirect-host cues, including reliability and detectability (Colazza *et al.* 1999, Conti *et al.* 2004, Peri *et al.* 2006, Salerno *et al.* 2009).

To uncover the foraging strategy of *A. orientalis* and its ability to locate infested patches with potential host eggs, bioassays were conducted to record and analyze parasitoid behavior when exposed to *L. delicatula* ‘footprints’ (chemical compounds deposited on substrates after passage of adult). Moreover, to better grasp the role of the ootheca and how the wasp responds to it, the sequential assessment, acceptance behavior, and parasitization of eggs by *A. orientalis* were evaluated when the parasitoids were exposed to covered versus uncovered egg masses.

Materials and Methods

Insects

Spotted lanternfly field mated adults were collected in November 2018, in Marsh Creek Park, Downingtown PA (40°03'52.9"N, 75°43'48.5"W), after citizens reported sighting the insect in this area. Specimens were transported to the USDA-ARS quarantine facility in Newark, DE, and maintained on field-collected *A. altissima* stems in growth chambers (24°C, 16:8 [L:D] h and 70% RH). Populations of the egg-parasitoid *A. orientalis* originally collected from *L. delicatula* egg masses in China by J. Gould were maintained on the same host in quarantine culture at the APHIS laboratory in Massachusetts and were shipped to the ARS laboratory in Delaware for these studies. *Lycorma delicatula* egg masses subjected to the egg assessment and choice-test experiments were collected in February 2019 from the same abovementioned location, thus were approximately 3 months old and possessed a hardened ootheca.

Foraging Behavior Bioassays

All experiments were carried out in the quarantine facility of USDA-ARS laboratories in Newark, DE, at $23 \pm 1^\circ\text{C}$ and $35 \pm 10\%$ RH. Step (1): a single gravid adult female *L. delicatula* was placed in an arena consisting of a petri dish, confined in a plastic container,

and forced to walk on a filter paper (4.25 cm \varnothing) for 30 min, to collect its chemical footprints. Whenever the insect rested and stopped walking, the container was gently rotated to motivate continued movement. Step (2): the contaminated filter paper was then transferred to a sterile glass petri dish (8 cm \varnothing) which was placed on a LED light pad (MEDALight LP-400N, Technical Lamps Ltd., Middlesex, United Kingdom), providing illumination from below to optimize visibility and contrast. Directly above the center of the light pad, a camera (ICD-49, Ikegami Tsushinki Co., Ltd., Tokyo, Japan) was mounted on a portable tripod and connected to a video monitor and desktop PC. Step (3): a single 2- to 4-d-old, mated, naive—had no previous experience of an egg mass—*A. orientalis* female was placed in the arena and confined under a plastic lid (0.5 cm height). Using the motion-tracking software EthoVision XT 8.0 (Noldus Information Technology, Wageningen, The Netherlands), the behavior of the wasp was recorded, tracked, and processed in real time (Fig. 3). The variables studied included the wasp’s residence time (time spent on the filter paper), mean linear velocity (mm/s), angular velocity ($^\circ/\text{s}$), and distance moved (mm). Recording commenced 2 s after the wasp was detected by the software on the filter paper and stopped 15 s after it left the paper. Each wasp was used for one replicate only, and 25 replicates were conducted for each treatment. A control treatment consisted of a noncontaminated filter paper. For both treatments, each filter paper was used for five replicates, and 25 replicates were conducted for each treatment. We tested for an effect of the order in which wasps were placed on the filter paper to see whether the footprints might have a diminishing effect with time or whether the previous presence of wasps walking on the filter paper might affect behavior of subsequent wasps. For all response variables except residence time, we used multiple regression models to test for a main effect of wasp sequence and for an interaction between sequence and treatment, after controlling for block. Because residence time did not meet the multiple regression assumption of normality under any transformation used, we performed a Kruskal–Wallis test to evaluate the effect of wasp sequence.

Egg Assessment and Acceptance

To decode the wasp’s ovipositional sequence and assess the role of the foam egg-covering (ootheca) in providing ovipositional signals or protection, the behavior of the wasp was observed and recorded

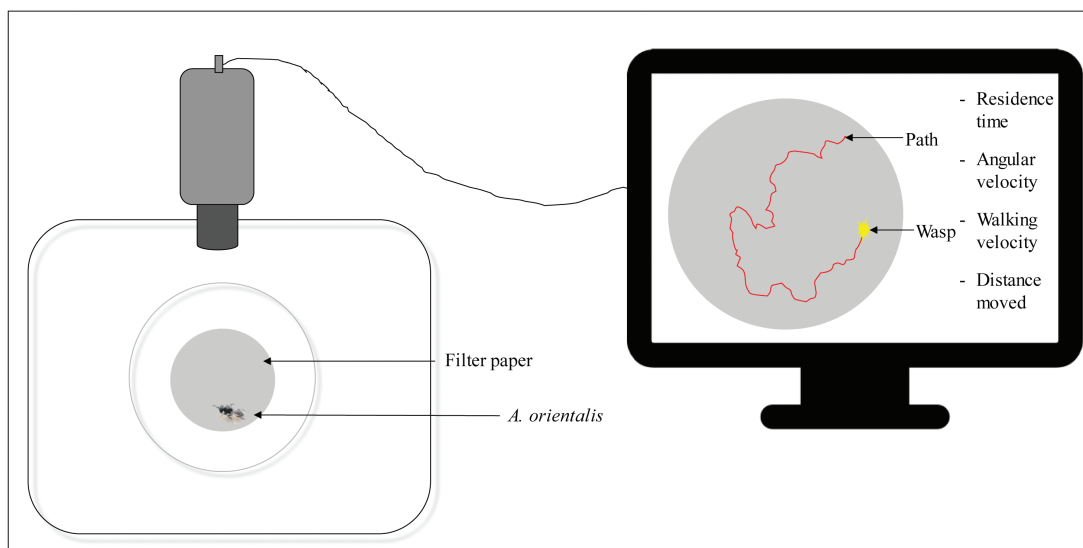


Fig. 3. Diagram of the experimental setup concerning the foraging behavior of the wasp, with the monitor displaying the recorded path and variables.

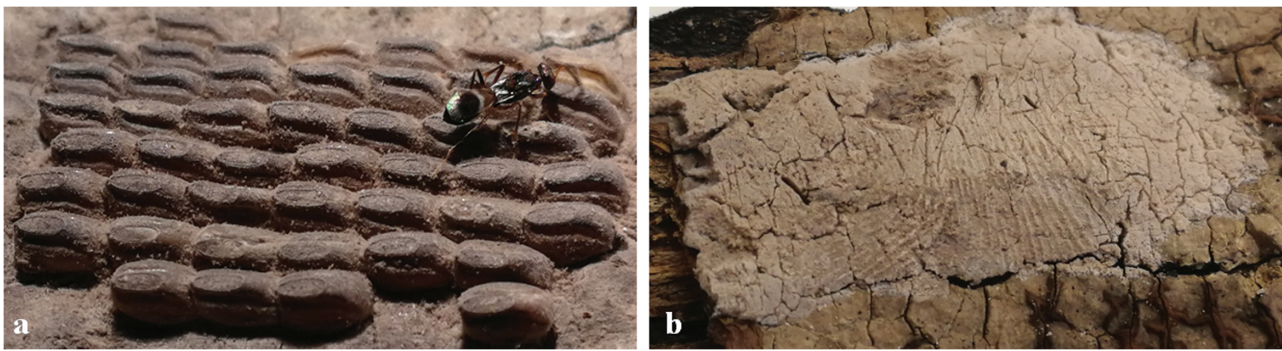


Fig. 4. A female *Anastatus orientalis* examining an uncovered egg mass (a). An intact egg mass within ootheca laid on a piece of bark (b).

on egg masses covered by *L. delicatula* secretions and on artificially uncovered ones (Fig. 4). The uncovering process consisted of brushing the egg mass with a fine brush for 1 min, the time needed to remove the foam covering and fully expose all underlying eggs. First, a subset of egg masses was presented to wasps, to identify the different steps leading to oviposition. The steps observed were as follows: 1) antennal contact—contact with the egg mass by at least one wasp antennae; 2) antennation and arrestment—wasp slows down its movement and intensifies antennation of the egg surface; 3) probing—insertion of the ovipositor in an egg for a brief period (0–2 min); and 4) oviposition/abdominal oscillation—insertion of the ovipositor in an egg for a long period (>9 min), with abdominal vibrations and oscillations probably corresponding to the deposition of an egg, as has been shown for Trichogrammatidae (Kaiser *et al.* 1987), Encyrtidae (Strand 1989), and Mymaridae (van Baaren *et al.* 1995). All egg masses in this experiment were on pieces of dry wood on which the eggs were naturally deposited in the field. The arena for observations consisted of an egg mass in a glass petri dish under a binocular microscope, where a single 4- to 5-d-old, mated, naive *A. orientalis* female was gently placed and confined by a plastic lid (0.5 cm height). Observations lasted for 25 min, starting when the wasp contacted the egg mass. Both the wasp and the egg mass were replaced for each replicate, with a total of 12 replicates for each treatment.

Parasitism Choice Test of Covered versus Uncovered Egg Masses

To follow-up on parasitoid ovipositional preferences, *A. orientalis* wasps were set up in a choice test with *L. delicatula* egg masses with an ootheca covering and without. Thirty replicates of one newly emerged (<24 h old) female and male *A. orientalis* were each placed in a rearing container (16-oz plastic deli cup with mesh lid). The wasps were given 1 wk pre-oviposition under ‘Beijing-conditions’ that cycled from 25 to 14°C daily with 13.5 h of light and 65% RH. They had honey streaked on the mesh lid. If any male died during this week, it was replaced. Following the 1 wk pre-oviposition, two *L. delicatula* egg masses were introduced to each cup, one with an intact ootheca, whereas the other had the ootheca brushed off, as previously described. Wasps were allowed to oviposit for 1 wk, then they were removed, and the egg masses were separated into two distinct cups to monitor subsequent emergence of *L. delicatula* nymphs and of *A. orientalis* progeny (emerged and unemerged wasps) from each type of egg mass independently. The eggs were maintained under ‘Beijing-conditions’ for 1 mo, then were moved to 25°C constant temperature and 16 h of light to encourage wasp emergence. Hatching of *L. delicatula* nymphs and *A. orientalis* wasps was counted, and unhatched eggs were dissected to determine their fate.

Statistical Analysis

For *A. orientalis* foraging behavior, the distributions of all response variable values for each treatment were evaluated for normality using a Shapiro Wilk test ($\alpha = 0.05$), and if required, appropriate transformations were made. The angular velocity ($P = 0.52$) and linear walking velocity ($P = 0.13$) data were normally distributed. The distance data were not normally distributed ($P < 0.001$); therefore, logarithmic transformation (\log_{10}) was applied. Residence time was not normally distributed under any transformation utilized, so a nonparametric test was used. Two-tailed Student’s *t*-tests were then used to compare the response of *A. orientalis* on footprint-contaminated filter papers and on controls, except for residence time, for which we utilized a Wilcoxon rank-sum test. For egg assessment and acceptance, the frequencies of each parasitoid behavior on covered and uncovered egg masses were compared using generalized linear models with binomial distributions. Regarding the parasitism choice test, differences in the number of wasp offspring in each type of egg mass (removed ootheca vs intact ootheca) were compared using a one-sided *t*-test. All statistical analyses were performed in R (v. 3.4.3).

Results

Foraging Behavior

The behavior of the wasps varied greatly on the treated substrate compared with the controls. Significant differences were observed between the two treatments regarding the residence time, distance moved, and linear walking velocity (Fig. 5). On average, the wasp’s residence time on footprint-contaminated filter papers was over fourfold (mean 318.6 ± 30.2 s SEM) than that spent on the control (65.6 ± 6.5 s) with ($W = 13$; $P < 0.0001$). The distance moved by the wasp was significantly greater on the footprint-contaminated filter paper (259.4 ± 29.7 cm) when compared with the control (73.7 ± 10.7 cm; $t_{48} = 7.34$; $P < 0.0001$). The linear walking velocity of *A. orientalis* was significantly slower on footprint-contaminated filter paper (11.6 ± 0.8 mm/s) compared with the control (18.65 ± 1.01 mm/s; $t_{48} = 5.28$; $P < 0.0001$). There was no significant difference between the angular velocity of the wasp on footprint-contaminated substrate ($115.2 \pm 4.7^\circ/\text{s}$) compared with controls ($108.3 \pm 6.7^\circ/\text{s}$; $t_{48} = 0.84$; $P > 0.402$). There was no main or interaction effect of the order in which wasps were placed on the filter papers ($P > 0.05$ for all response variables).

Egg Assessment and Acceptance

In total, 12 egg masses were presented to 12 unique *A. orientalis* females for each treatment. Our results summarized (Table 1) reveal

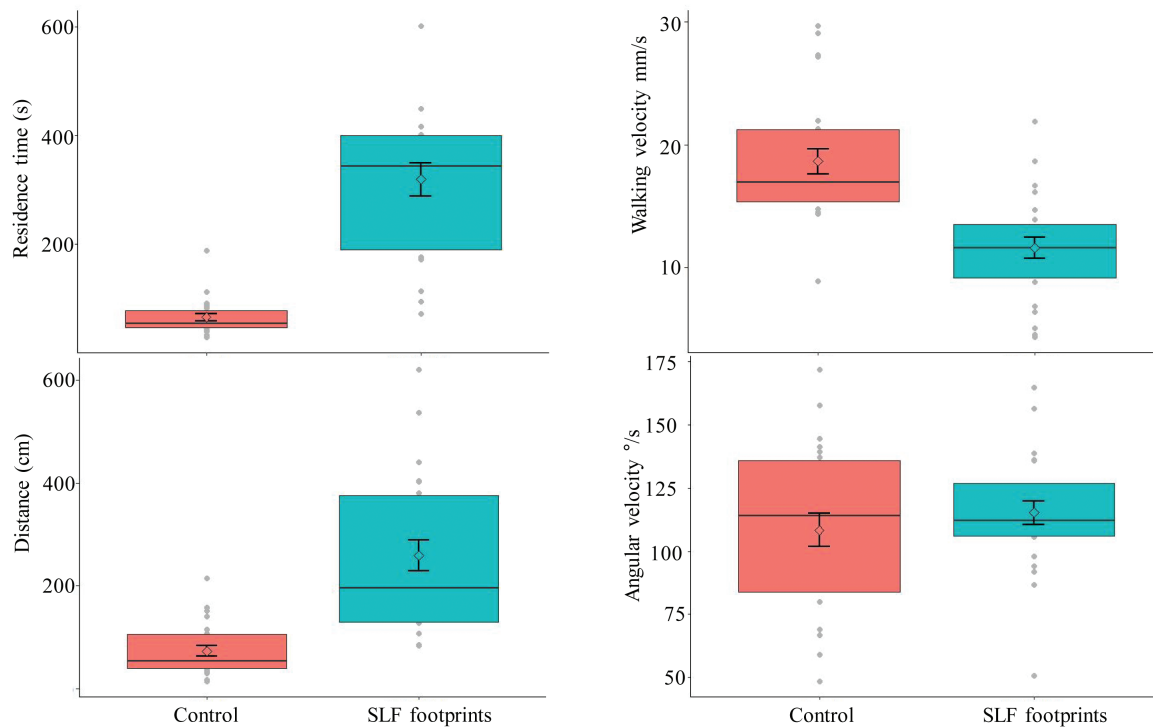


Fig. 5. Boxplots showing residence time, linear walking velocity, distance covered, and angular velocity of *Anastatus orientalis* on footprint-contaminated substrate and on control. The first three of these recorded variables were statistically different from controls, whereas only angular velocity was not. Boxes represent interquartile range with horizontal bar as median. Diamonds and whiskers indicate mean \pm SEM. Gray dots represent data points that fall outside the interquartile range. $n = 25$ replicate per treatment.

Table 1. Ovipositional sequence, egg assessment, and acceptance by *Anastatus orientalis* of *Lycorma delicatula* covered and uncovered egg masses

SLF egg mass	Contact	Antennation/ arrestment	Probing	Oviposition/ oscillation
Covered	12	12	8*	7*
Uncovered	12	12	2	2

$n = 12$.

* $P < 0.05$.

that for both treatments, all wasps encountered the egg mass and exhibited an arrestment behavior, characterized by a slower motion and intense antennation of the substrate. Covered egg masses were probed with significantly higher frequency (66.6%) compared with uncovered ones (16.6%; $P = 0.0197$). Inferred oviposition instances were also significantly more frequent on covered egg masses (58.3%) rather than on uncovered ones (16.6%; $P = 0.0451$).

Parasitism Choice Test of Covered versus Uncovered Egg Masses

Significant differences were observed between the two treatments, with fewer wasp progeny produced in egg masses that had the ootheca covering removed (17.57 ± 2.05), compared with egg masses with the ootheca left intact (23.63 ± 2.18 ; $t_{37.7} = -2.0253$, $P = 0.0237$).

Discussion

Insect parasitoids locate their hosts through sequences of behavioral steps that are mainly mediated by chemical cues that can be directly or indirectly related to the host (Vinson 1998). On control substrates

(noncontaminated filter papers), female *A. orientalis* residence times were short, they walked rapidly, and they consequently explored only small sections of the substrate. The parasitoid reacted to chemical footprints deposited by *L. delicatula* gravid females by altering orthokinetic (i.e., speed of movement dependent on the intensity of the stimulus) and locomotory behavior, and clearly initiated an arrestment response. The latter is characterized by a decrease in linear walking velocity, and an increase in residence time and distance covered on footprint-contaminated substrate (Peri *et al.* 2006, Rostás and Wölfling 2009, Konopka *et al.* 2018). For scelionid egg parasitoids, an increase in angular velocity (directional change) is also associated with an arrestment response (Colazza *et al.* 1999); however, this was not observed for *A. orientalis*, perhaps due to its larger size compared with scelionids. Nonetheless, the wasps exhibited an intensified searching behavior, prolonging their residence time and slowly covering greater distances on patches containing cues that might lead them to their host eggs.

Our results suggest that cuticular hydrocarbons or other chemicals from *L. delicatula* might be deposited on the substrate where the insect walked, potentially through its tarsal adhesive pads, or via abdominal contact. Consequently, *A. orientalis* switches to a state of 'motivated searching' (Vinson 1998), restricting the search to an area where eggs are more likely to be found. The *L. delicatula* females tested in the present study were gravid, so the wasps may have been detecting cues more closely proximate to oviposition. Salerno *et al.* (2009) found that *Trissolcus brochymenae* Ashmead (Hymenoptera: Scelionidae) residence time on substrate contaminated by mated *Murgantia histrionica* Hahn (Hemiptera: Pentatomidae) female footprints was significantly longer than that of mated males and unmated males and females. Further investigations of mated and unmated *L. delicatula* females and males are needed to better understand this aspect of wasp foraging.

Peri *et al.* (2013) showed that *Trissolcus* egg parasitoids only responded positively to footprints of hosts they are normally associated with. For instance, *T. brochymenae* only responded to footprints of *M. bistrionica*, not to those of *Graphosoma semipunctatum* Fabricius (Hemiptera: Pentatomidae) or to those of *Nezara viridula* L. (Hemiptera: Pentatomidae). In contrast, *Trissolcus basalis* Wollaston (Hymenoptera: Scelionidae) only responded to footprints of its host *N. viridula* and not to the other heteropteran species tested. Insect host–parasitoid interactions are shaped by natural selection (Godfray 1994); thus, the strength of *A. orientalis* arrestment on *L. delicatula* footprints may be considered a result of a pairwise coevolution and warrants further testing.

Concentrated egg depositions in the form of oothecae (sometimes referred to as egg cases or egg pods) are common among dictyopterans (cockroaches, termites, and praying mantises), orthopterans, and even some coleopterans (Grimaldi and Engel 2005). Oothecae probably evolved to protect the eggs from desiccation, predators, and parasitoids (Salas-Araiza *et al.* 2013). Our study revealed that *A. orientalis* probed and oviposited more frequently through the ootheca, rather than in artificially uncovered egg masses. This suggests that both mechanical, and chemical cues present in the ootheca may serve as triggers for probing and oviposition. For example, Vinson and Piper (1986) found that *Tetrastichus hagenowii* Ratzeburg (Hymenoptera: Eulophidae) relies on mucopolysaccharides and calcium oxalates as kairomones for the recognition of its host *Periplaneta americana* L. (Blattodea: Blattidae), but only when these cues are present on curved surfaces. Thus, the absence of an ootheca in our uncovered egg masses may have been suboptimal for host recognition and acceptance by *A. orientalis*. Although not systematically investigated here, it is worth mentioning that throughout our observations of parasitoids presented with uncovered eggs, several instances were recorded where the wasps probed the wood on which the eggs were laid, even after having antennated the exposed eggs (Fig. 6).

It might be that while *A. orientalis* adults are chewing their way out of *L. delicatula* ootheca, they ingest or acquire chemical cues that later influence their ovipositional preferences. Hopkins' host selection principle states that phytophagous and parasitic insects will prefer to continue to breed on hosts onto which they had been laid and developed (Hopkins 1916, 1917). This notion was refined and expanded—and also heavily criticized in later studies, as reviewed by Barron (2001). Corbet (1985) proposed that adults may respond to certain chemical cues acquired in the larval environment during early imaginal stages, termed 'chemical legacy'.



Fig. 6. *Anastatus orientalis* female probing the wooden plant material onto which *Lycorma delicatula* egg masses were deposited, with ovipositor clearly inserted in nonhost tissue.

This host-associated learning occurs as the parasitoid emerges from the host or shortly after emergence (Giunti *et al.* 2015). The adult females *A. orientalis* used in the present study had emerged from egg masses with intact oothecae, and this may have influenced host preference.

Our parasitism choice test revealed that *L. delicatula* eggs contained within an ootheca are significantly more suitable than uncovered egg masses for *A. orientalis* successful parasitism and development. *Anastatus orientalis* enters diapause and overwinters as mature larvae in *L. delicatula* egg masses (Hou 2013). The preference for eggs within ootheca indicates that not only is the wasp using its host for reproduction but could also be benefiting from the egg case for the protection of its own progeny from harsh winter conditions. An arms race metaphor might be appropriate in this context, with *A. orientalis* managing to circumvent the structural defense of *L. delicatula* ootheca to its own likely fitness gains, shedding light on the potential coevolution between these two species. The consequences of such a behavior on the likelihood of effective control by *A. orientalis* require further investigation and field validation.

Our results provide insight into the foraging behavior of *A. orientalis*, a potential candidate for classical (importation) biological control of invasive *L. delicatula*. The former perceives chemical footprints of *L. delicatula* and invests vital time and energy in searching for host eggs when these traces are present. Further studies on the identity of the compounds eliciting such responses may be helpful in designing future parasitoid surveys. Future attention to the wasp's response to long-range cues and behavior in complex natural environments is also well deserved. The preferences of the wasp are no less adaptive than its host's defensive strategies, with eggs in oothecae more frequently probed and stung than exposed ones. This warrants further chemical analyses on the composition of the oothecal covering to better understand its role and function. The higher parasitism frequency of egg masses with oothecae may inform and improve rearing methods of this biological control agent. Future studies on the host range and life cycle of this wasp will improve our understanding of its host specificity and ecological risk assessment for its potential introduction against this newly emerging invader. This study was made possible through access to biosecurity containment facilities and foreign exploration to gather natural enemies of *L. delicatula* in its native range. Early initiation of investigations aimed toward importation biological control provide valuable ecological insights, which can inform integrated pest management tactics, irrespective of whether a safe agent is successfully screened and released.

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References Cited

- Barron, A. B. 2001. The life and death of Hopkin's host selection principle. *J. Insect Behav.* 6: 725–737.
- Choi, M. Y., Z. Q. Yang, X. Y. Wang, Y. L. Tang, Z. R. Hou, J. H. Kim, and Y. W. Byeon. 2014. Parasitism rate of egg parasitoid *Anastatus orientalis* (Hymenoptera: Eupelmidae) on *Lycorma delicatula* (Hemiptera: Fulgoridae) in China. *Korean J. Appl. Entomol.* 53: 135–139.
- Clifton, E. H., L. A. Castrillo, A. Gryganskyi, and A. E. Hayek. 2019. A pair of native fungal pathogens drives decline of a new invasive herbivore. *Proc. Natl. Acad. Sci. USA* 116: 9178–9180.
- Colazza, S., G. Salerno, and E. Wajnberg. 1999. Volatile and contact chemicals released by *Nezara viridula* (Heteroptera: Pentatomidae) have a kairomonal effect on the egg parasitoid *Trissolcus basalisi* (Hymenoptera: Scelionidae). *Biol. Control* 16: 310–317.
- Conti, E., G. Salerno, F. Bin, and S. B. Vinson. 2004. The role of host semi-chemicals in parasitoid specificity: a case study with *Trissolcus brochymenae* and *Trissolcus simoni* on pentatomid bugs. *Biol. Control* 29: 435–444.
- Corbet, S. A. 1985. Insect chemosensory responses: a chemical legacy hypothesis. *Ecol. Entomol.* 10: 143–153.
- Dara, S. K., L. Barringer, and S. P. Arthurs. 2015. *Lycorma delicatula* (Hemiptera: Fulgoridae): a new invasive pest in the United States. *J. Integr. Pest Manag.* 6: 20.
- (EPP0) European Plant Protection Organization. 2019. EPP0 Global Database. (<https://www.eppo.int>) (accessed 22 March 2019).
- Fatouros, N. E., M. Dicke, R. Mumm, T. Meiners, and M. Hilker. 2008. Foraging behavior of egg-parasitoids exploiting chemical information. *Behav. Ecol.* 19: 677–689.
- Giunti, G., A. Canale, R. H. Messing, et al. 2015. Parasitoid learning: current knowledge and implications for biological control. *Biol. Control* 90:208–219.
- Godfray, H. C. J. 1994. Parasitoids: behavioral and evolutionary ecology. Princeton University Press, Princeton, NJ.
- Grimaldi, D., and M. S. Engel. 2005. Evolution of the insects. Cambridge University Press, New York.
- Han, J. M., H. Kim, E. J. Lim, S. Lee, Y. J. Kwon, and S. Cho. 2008. *Lycorma delicatula* (Hemiptera: Auchenorrhyncha: Fulgoridae: Aphaeninae) finally, but suddenly arrived in Korea. *Entomol. Res.* 38: 281–286.
- Hilker, M., and N. E. Fatouros. 2015. Plant responses to insect egg deposition. *Annu. Rev. Entomol.* 60: 493–515.
- Hopkins, A. D. 1916. Economic investigations of the scolytid bark and timber beetles of North America. U.S. Department of Agriculture Program of Work for 1917. p. 353.
- Hopkins, A. D. 1917. A discussion of C. G. Hewitt's paper on "Insect Behaviour". *J. Econ. Entomol.* 10: 92–93.
- Hou, Z. R. 2013. Study on the *Lycorma delicatula* and egg parasitoids. M.S. thesis dissertation, Chinese Academy of Forestry, Beijing, China.
- Kaiser, L., M. Pham-Delegue, N. Hawlitzki, and C. Masson. 1987. Comportement de recherche et d'oviposition de *Trichogramma maidis* Pint. et Voeg. (Hym: Trichogrammatidae): analyse quantitative en conditions contrflres. *C. R. Acad. Sci. Paris* 305: 95–99.
- Kang, C. K., S. I. Lee, and P. G. Jablonski. 2011. Effect of sex and bright coloration on survival and predator-induced wing damage in an aposematic lanternfly with startle display. *Ecol. Entomol.* 36: 709–716.
- Kang, C., H. Moon, T. N. Sherratt, S. I. Lee, and P. G. Jablonski. 2016. Multiple lines of anti-predator defence in the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae). *Biol. J. Linn. Soc.* 120: 115–124.
- Kim, J. G., E. H. Lee, Y. M. Seo, and N. Y. Kim. 2011. Cyclic behavior of *Lycorma delicatula* (Insecta: Fulgoridae) on host plants. *J. Insect Behav.* 24: 423–435.
- Kim, H., M. Kim, D. H. Kwon et al. 2013. Molecular comparison of *Lycorma delicatula* (Hemiptera: Fulgoridae) isolates in Korea, China, and Japan. *J. Asia-Pac. Entomol.* 16: 503–506.
- Konopka, J. K., D. Poinapen, T. Garipey, and J. N. McNeil. 2018. Understanding the mismatch between behaviour and development in a novel host-parasitoid association. *Sci. Rep.* 8: 15677.
- Laing, J. 1937. Host-finding by insect parasites. I. Observations on the finding of hosts by *Alysia manducator*, *Mormoniella vitripennis* and *Trichogramma evanescens*. *J. Anim. Ecol.* 6: 298–317.
- Liu, M. and J. Mottern. 2017. An old remedy for a new problem? Identification of *Ooencyrtus kuwanae* (Hymenoptera: Encyrtidae), an egg parasitoid of *Lycorma delicatula* (Hemiptera: Fulgoridae) in North America. *J. Insect Sci.* 17: 1–6.
- Leach, H., D. J. Biddinger, G. Krawczyk, E. Smyers, and J. M. Urban. 2019. Evaluation of insecticides for control of the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae), a new pest of fruit in the Northeastern U.S. *Crop Prot.* 124: 104833.
- Lee, J. E., S. R. Moon, H. G. Ahn, S. R. Cho, J. O. Yang, C. Yoon, and J. H. Kim. 2009. Feeding behavior of *Lycorma delicatula* (Hemiptera: Fulgoridae) and response on feeding stimulants of some plants. *Korean J. Appl. Entomol.* 48: 467–477.
- Lee, D. H., Y. L. Park, and T. C. Leskey. 2019. A review of biology and management of *Lycorma delicatula* (Hemiptera: Fulgoridae), an emerging global invasive species. *J. Asia-Pac. Entomol.* 22: 589–596.
- Liu, H., K. A. Hoelmer, and J. S. Gould. 2017. Natural enemies of the spotted lanternfly in Asia and North America. USDA Interagency Forum on Invasive Species. (<https://www.ars.usda.gov/research/publications/publication/?seqNo115=338695>) (accessed 25 February 2019).
- Park, J. D., M. Y. Kim, S. G. Lee, S. C. Shin, J. H. Kim, and I. K. Park. 2009. Biological characteristics of *Lycorma delicatula* and the control effects of some insecticides. *Korean J. Appl. Entomol.* 48: 53–57.
- Pennsylvania Department of Agriculture. 2018. Spotted lanternfly. (<https://extension.psu.edu/spotted-lanternfly>) (accessed 13 March 2019).
- Peri, E., M. A. Sole, E. Wajnberg, and S. Colazza. 2006. Effect of host kairomones and oviposition experience of an egg parasitoid. *J. Exp. Biol.* 209: 3629–3635.
- Peri, E., F. Frati, G. Salerno, E. Conti, and S. Colazza. 2013. Host chemical footprints induce host sex discrimination ability in egg parasitoids. *PLoS One* 8: e79054.
- Rostás, M., and M. Wölfling. 2009. Caterpillar footprints as host location kairomones for *Cotesia marginiventris*: persistence and chemical nature. *J. Chem. Ecol.* 35: 20–27.
- Salas-Araiza, M. D., W. P. Mackay, J. Valdez-Carrasco, E. Salazar-Solis, and O. A. Martínez-Jaime. 2013. Characterization and comparison of the eggs of seven species of Mexican grasshoppers. *Southwest. Entomol.* 38: 267–274.
- Salerno, G., F. Frati, E. Conti, C. De Pasquale, E. Peri, and S. Colazza. 2009. A finely tuned strategy adopted by an egg parasitoid to exploit chemical traces from host adults. *J. Exp. Biol.* 212: 1825–1831.
- Spears, L. R., and A. M. M. Mull. 2019. Spotted lanternfly [*Lycorma delicatula* (White)]. Utah State University Extension and Utah Plant Pest Diagnostic Laboratory. UPPDL, Logan, Utah.
- Strand, M. R. 1989. Oviposition behavior and progeny allocation of the polyembryonic wasp *Copidosoma floridanum* (Hymenoptera: Encyrtidae). *J. Insect Behav.* 2: 355–369.
- van Baaren, J., J. P. Nénon, and G. Boivin. 1995. Comparison of oviposition behavior of a solitary and a gregarious parasitoid (Hymenoptera: Mymaridae). *J. Insect Behav.* 8: 671–686.
- Vet, L. E. M., and M. Dicke. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.* 37: 141–172.
- Vinson, S. B. 1998. The general host selection behavior of parasitoid Hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. *Biol. Control* 11: 79–96.
- Vinson, S. B., and G. L. Piper. 1986. Source and characterization of host recognition kairomones of *Tetrastichus hagenowii*, a parasitoid of cockroach eggs. *Physiol. Entomol.* 11: 459–468.
- Yang, C. 1994. Descriptions of *Dryinus lycormae* sp. n. and its biological notes (Hymenoptera Dryinidae), pp. 37–42. In Shen X. and Shi Z. (eds.), Fauna and taxonomy of insects in Henan, 1. China Press Agricultural Science and Technology, Beijing.
- Yang, Z. Q., W. Y. Choi, L. M. Cao, X. Y. Wang, and Z. R. Hou. 2015. A new species of *Anastatus* (Hymenoptera: Eupelmidae) from China, parasitizing eggs of *Lycorma delicatula* (Homoptera: Fulgoridae). *Zool. Syst.* 40: 290–302.