United States Department of Agriculture

30th USDA Interagency Research Forum on Invasive Species

Urban Island Invasion

Annapolis, MD | January 14-17, 2020

US Forest Service

Forest Health Assessment & Applied Sciences Team

FHTET-2020-01
August 2020

NEW FINDINGS ON SPOTTED LANTERNFLY BEHAVIOR, BIOLOGY, AND SURVEY TOOLS

Miriam F. Cooperband

USDA APHIS PPQ S&T, 1398 West Truck Rd., Buzzards Bay, MA 02542

ABSTRACT

Spotted lanternfly, SLF, *Lycorma delicatula*, is a polyphagous, phloem-feeding invasive fulgorid native to China. It was discovered in Pennsylvania in 2014 and has now spread to a number of neighboring states. This damaging pest feeds on a broad range of over 100 host plants, but its preferred host, tree-of-heaven, *Ailanthus altissima*, another invasive from China, is broadly distributed throughout the US. SLF can seriously damage or even kill grape vines, and threatens a range of other commodities such as fruit trees, nursery stock, and timber[1]. It has been found to accumulate in great numbers on numerous species of plants, particularly on tree-of-heaven[2]. Our main objective has been to develop semiochemical lures for detection and survey, and possibly control. As such, we have investigated both kairomones and pheromones of SLF. This overview highlights some of our work from 2018 and 2019.

Kairomones. We found that in the Y-tube, SLF quite often were significantly attracted to volatiles produced by a number of plant species when compared to a blank control. However, when offered a choice between tree-of-heaven volatiles and other plant volatiles, tree-of-heaven was either significantly more attractive than the alternative (milkweed, staghorn sumac, hops, wild grape, chinaberry, Norway spruce) or there was no significant difference (black cherry, tulip tree, alfalfa, black walnut, horseradish,

Table 1. Presence of EAD active and/or behaviorally active compounds in 17 plant species.

15 ACTIVE COMPOUNDS	Ailanthus	Wild Grape	Hops	Black Locust	Black Walnut	Virginia Creeper	Oriental Bittersweet	Staghorn Sumac	Black Cherry	Red Oak	Chinaberry	Spicebush	Tulip Tree	Red Maple	Apple	Black Birch	Norway Spruce	TOTAL
(E,E)-α-farnesene	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	17
ß-caryophyllene	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	17
ß-ocimene	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			15
methyl salicylate	x	x	x	x	x	x	x	x	x	x	x	x		x	x			14
(E)-4,8-dimethyl-1,3,7-nonatriene	x	x	x	x	x	x	x	x	x	x	x	x	x			x		14
(Z)-3-hexenyl acetate	x	x	x	x	x	x	x		x	x	x			x		x		12
linalool	x	x	x	x		x		x	x		x	x	x				x	11
ß-ylangene	x	x	x	x	x	x	x	x		x			x					10
sulcatone	x	x		x					x	x		x						6
(Z)-3-hexenol	x			x	x					x		x						5
geranylacetone	x							x	x		x							4
methyl benzoate	x	x					x	x										4
(Z)-3-hexenyl butyrate		x	x			x					x							4
1-octen-3-ol	x		x				x											3
4-terpineol					x												x	2
TOTAL	13	11	10	10	9	9	9	9	9	9	9	8	6	5	4	4	4	

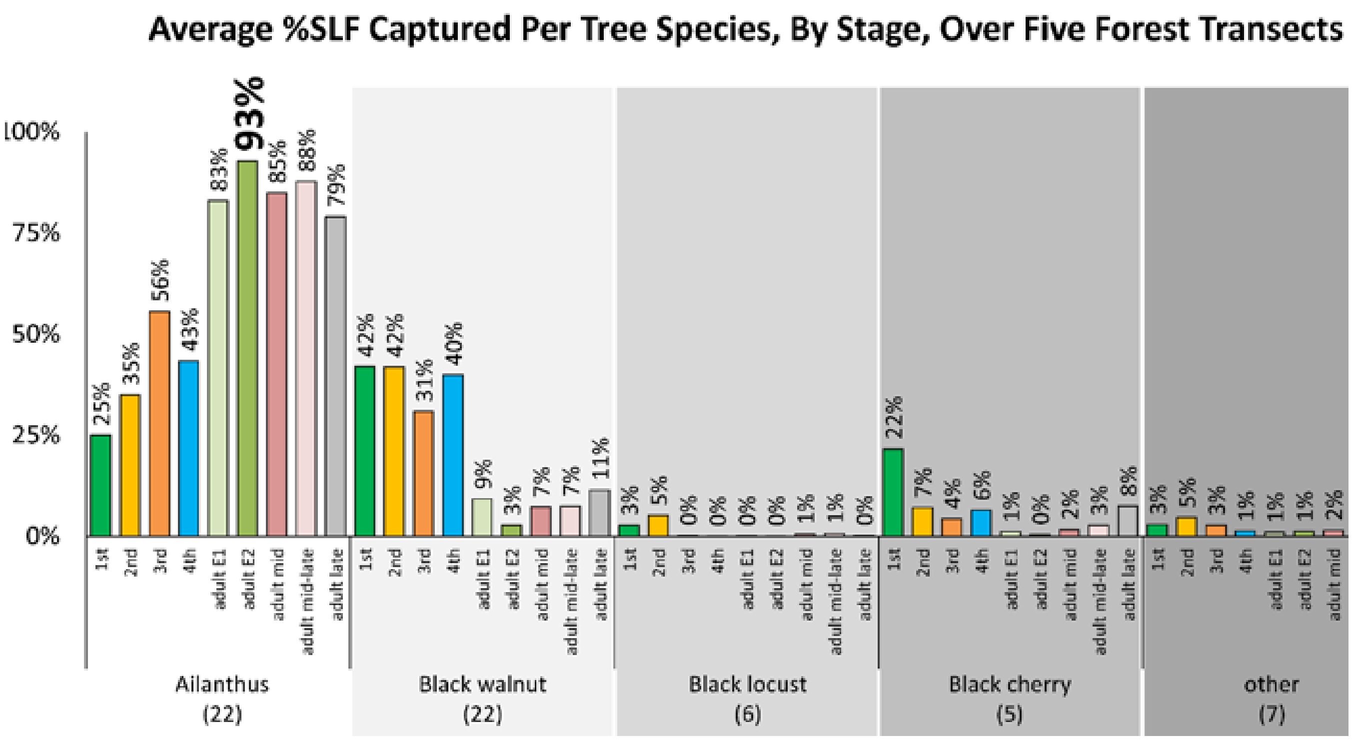
oriental bittersweet). The alternative plant volatiles were never significantly more attractive than tree-of-heaven volatiles. We conducted gas chromatography coupled with electroantennographic detection (GC-EAD) using volatiles from tree-of-heaven and several other plant species, identified a number of EAD active compounds, and tested these synthetic compounds for attraction in the Y-tube, many of which were attractive. We compared volatile profiles of 17 plant species and found that tree-of-heaven contained the largest number of these active compounds. Other highly attractive plants like wild grape, hops, black locust, and black walnut also had profiles with a large number of these compounds (**Table 1**). Norway spruce was tested because it is not attractive to SLF, and it had the least of these compounds in its volatile profile.

Phenology. In order to investigate the presence of a pheromone, we first had to understand the phenology of SLF and when mating and other physiological states were taking place. We investigated the phenology of adults in 2016, 2017, 2018, and 2019. We found that adults go through four distinct periods which we define as follows (with approximate dates based on data from 2017 and 2018):

- Early 1 (feeding – Aug. 1-24)
- Early 2 (feeding and skewed sex ratios – Aug. 25 – Sept. 13)
- Mid (mating – defined by the first observation of mating in the field – Sept. 14-30)
- Late (oviposition – defined by the first appearance of fresh eggs – Oct. 1 until death)

In 2018, we trapped for SLF on all trees along five transects and found that SLF populations in these transects concentrated increasingly on tree-of-heaven with each developmental stage. This peaked with 93% of the SLF population on trees in transects concentrating on tree-of-heaven during the two weeks prior to mating (**Figure 1**). This suggests that tree-of-heaven is important in locating mates.

Figure 1. Phenology of host preference over five forest transects shwoing average percent of SLF captured per tree species, by stage (N trees total).



Pheromones. In 2018 and 2019, we conducted investigations to understand the role chemical communication plays in SLF forming aggregations both as nymphs and adults, as well as aggregations of predominantly one sex or the other. We started with a mark-release-recapture field study in 2018 in a low SLF density area, and released SLF on the ground so that substrate vibrations could be ruled out. We found that trees "baited" with groups of adult SLF attracted significantly more marked-released SLF than control trees, suggesting the use of SLF-produced or induced semiochemicals in order to aggregate[3].

We subsequently investigated what volatiles are produced and when, and who is attracted to whom at different adult stages. In our search for sex and/or aggregation pheromones, we identified a number of attractive compounds generated by SLF, and started constructing a "semio-phenology", in other words, a timeline of when different volatiles are produced or are attractive.

In 2019, every week starting from the emergence of first instar nymphs until the beginning of oviposition in October, we collected (1) SLF body extracts (adults in 2018, also), (2) SLF honeydew, and (3) headspace from live SLF on tree-of-heaven and tree-of-heaven without SLF as controls. The third study is still undergoing analysis, however, here we report findings so far from the first two approaches.

Body extracts – Whole body solvent extracts contained enormous amounts of cuticular waxes and hydrocarbons relative to volatile components, precluding their injection into the gas chromatograph for electroantennographic detection (GC-EAD) or mass spectrometry (GC-MS). However, these extracts were tested for attraction by all stages of SLF in a dual-choice olfactometer to determine who is attracted to whom, and when. Nymphs were attracted to the whole body extracts of other nymphs, suggesting that an aggregation pheromone may exist for nymphs. We found that males were attracted to extracts from females prior to mating, but not males, suggesting a sex pheromone may also be involved. Females were not attracted to female extracts, but just after emergence, females were attracted to male extracts (**Table 2**).

Table 2. Summary of attraction of SLF to volatiles from SLF body or SLF honeydew at different life stages. Each mark represents a series of insects tested against the volatile vs control in the dual choice olfactometer (Chi square goodness-of-fit test, average N = 24, α=0.05).

SUMMARY OF ATTRACTION

		Male Volatiles				Female Volatiles				Male Volatiles				Female Volatiles			
Volatile source:		BODY								HONEYDEW							
		E1	E2	M	L	E1	E2	M	L	E1	E2	M	L	E1	E2	M	L
Males are (✓) / not(×) attracted to:	Early-1	×				✓				✓				×			
	Early-2	×	××			✓	✓✓				✓×				××		
	Mid		×				✓×	×				✓				×	
	Late						×	×	×								
Females are (✓) / not(×) attracted to:	Early-1	✓				×				×				×			
	Early-2	×	××			×	××				××				✓×		
	Mid		×				××					×				×	
	Late						×	×	×								

Honeydew volatiles – Honeydew was collected every week and tested for attraction to fourth instars and adults in the olfactometer. Fourth instars were attracted to the volatiles emanating from their own honeydew. Interestingly, adult male and female SLF were only attracted to volatiles from their *own* honeydew, but not to honeydew from the opposite sex (**Table 2**).

This peculiar discovery may help to explain the skewed sex ratios we have observed and documented in the field every year since we started studying SLF [2, 4, 5]. Although we still do not understand why males and females sometimes aggregate in groups of mostly their own sex, this may provide an explanation as to how they find each other to form these aggregations.

Novel attractive compounds – Although the abundance of compounds in volatile collections was too low to elicit antennal responses in GC-EAD, over 30 compounds were identified (or tentatively identified) using GC-MS. The synthetic versions of 26 of these compounds were purchased or synthesized and screened using GC-EAD, and 12 were found to be antennally active. In addition, eight of these compounds were tested and found to be attractive in the dual-choice olfactometer. Strong antennal responses and attraction occurred in response to benzyl acetate and 2-nonanone. Also of interest was 2-heptanone, which was attractive to males, and 2-octanone, which was attractive to females. These three ketones were found in both honeydew and body volatiles, whereas benzyl acetate was found only in the honeydew. That 2-heptanone and 2-octanone were attractive to opposite sexes could reveal a mechanism for the skewed sex ratios observed on tree trunks prior to mating[4]. The purpose and evolutionary advantage of this observed sex ratio shift prior to mating is not yet understood. Initial field tests were inconclusive and more field studies are planned.

Acknowledgements.
This work was conducted through collaboration, coordination, and teamwork between many USDA personnel and university cooperators. University cooperators include Daniel Carrillo, University of Florida (UF), Matthew Wallace, East Stroudsburg University (ESU), and Ann Ray, Xavier University, (XU). Nathan Derstine (UF) conducted GC-EAD, volatile collections and chemical identification in search of kairomones. Linnea Meier (UF) conducted GC-EAD, volatile collections, chemical identification, and the mark-release-recapture study in search of pheromones. Isaiah Canlas (UF/XU) conducted dual choice olfactometer bioassays. Rearing was conducted by Isaiah Canlas and Sam Stella (USDA/XU). Kelly Murman (USDA/ESU) and Stefani Cannon (USDA/ESU) led a field crew and conducted field work as well as field volatile collections and catching SLF for experiments. USDA technicians in the field and lab were Leslie Abreu, Zachary DeSantis, Kerry Handelong, Kyle Kaye, Daniel Rinkenberg, and Chris Smith. Tappey Jones (Virginia Military Institute) synthesized compounds that were not commercially available.

REFERENCES

1. Baker TC, Smyers E, Urban J, Meng Z, Damadaram KP, Myrick AJ, Cooperband M, Domingue M. Progression of seasonal activities of adults of the spotted lanternfly, *Lycorma delicatula*, during the 2017 season of mass flight dispersal behavior in eastern Pennsylvania. Journal of Asia-Pacific Entomology. 2019; 22 (3): 705-13.
2. Cooperband MF, Meier LR, Canlas I, Murman K, Cannon S, Stella S, Smith C, Abreu L, Wallace MS, Carrillo D, Ray AM. Phenological studies on spotted lanternfly host plants, adult biology, and chemical ecology. 2019. pp. 56-58. *In*: Trepanowski N, Vieira K, Heller S, Booth E (eds.). Otis Laboratory 2018 Annual Report. United States Department of Agriculture, Buzzards Bay, MA. 99 pp.
3. Cooperband M, Meier L, Wickham J, Murman K, Cannon S, Abreu L. Tree effects: Understanding what makes "hot" trees hot to spotted lanternfly. 2019. pp. 62-63. *In*: Trepanowski N, Vieira K, Heller S, Booth E (eds.). Otis Laboratory 2018 Annual Report. United States Department of Agriculture, Buzzards Bay, MA. 99 pp.
4. Domingue MJ, Cooperband MF, Baker TC. Skewed adult sex ratios observed early in the North American

invasion of *Lycorma delicatula* (Hemiptera: Fulgoridae). Journal of Asia-Pacific Entomology. 2019: https://doi.org/10.1016/j.aspen.2019.11.002.

5. Francese JA, Cooperband MF, Murman KM, Cannon SL, Booth EG, Devine SM, Wallace MS. Developing traps for the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae). Environ Entomol. 2020; nvz166: https://doi.org/10.1093/ee/nvz166.