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# **Otis Laboratory Accomplishments 2018**

**Buzzards Bay MA  
Salinas CA • Bethel OH • East Stroudsburg PA**

**United States Department of Agriculture Animal and Plant  
Health Inspection Service Plant Protection and Quarantine**

**Science and Technology**

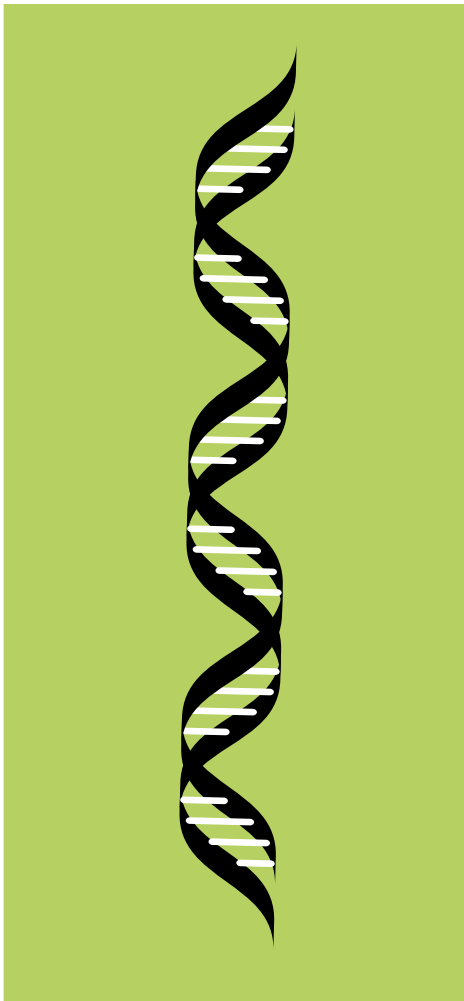
**Center for Plant Health Science and Technology**





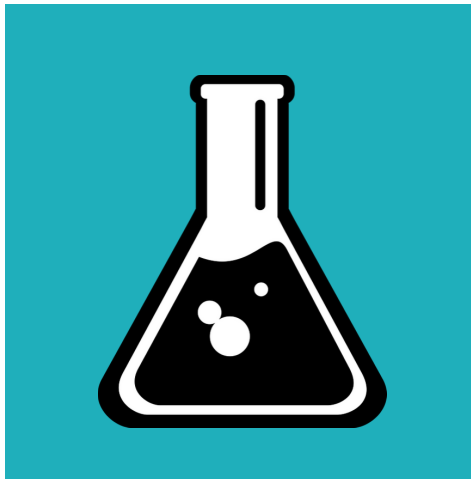
# OTIS

## LABORATORY



# 2018

## ACCOMPLISHMENTS



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This report was edited by: Nevada Trepanowski, Kendra Vieira, Sarah Heller, and Everett Booth

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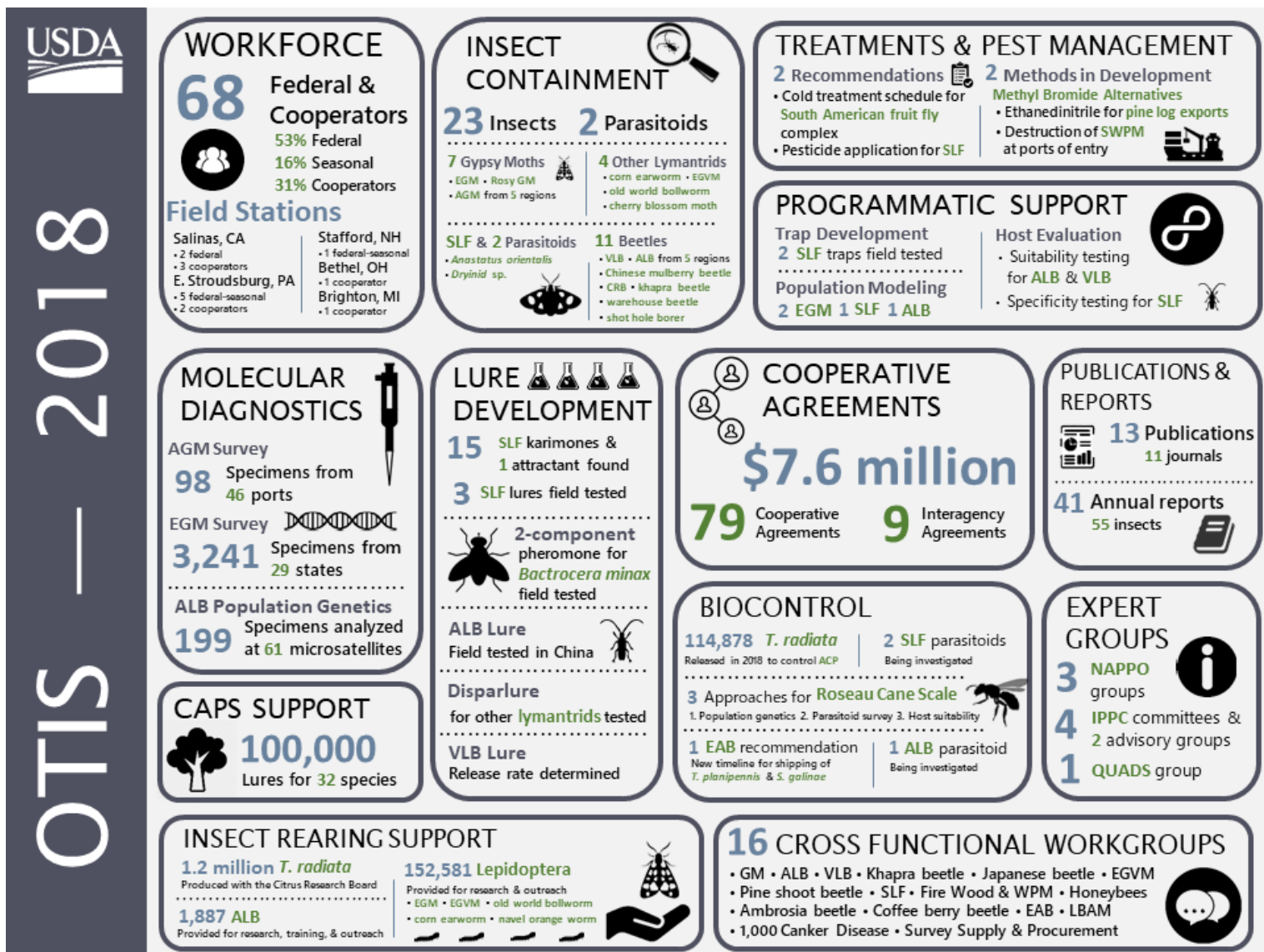
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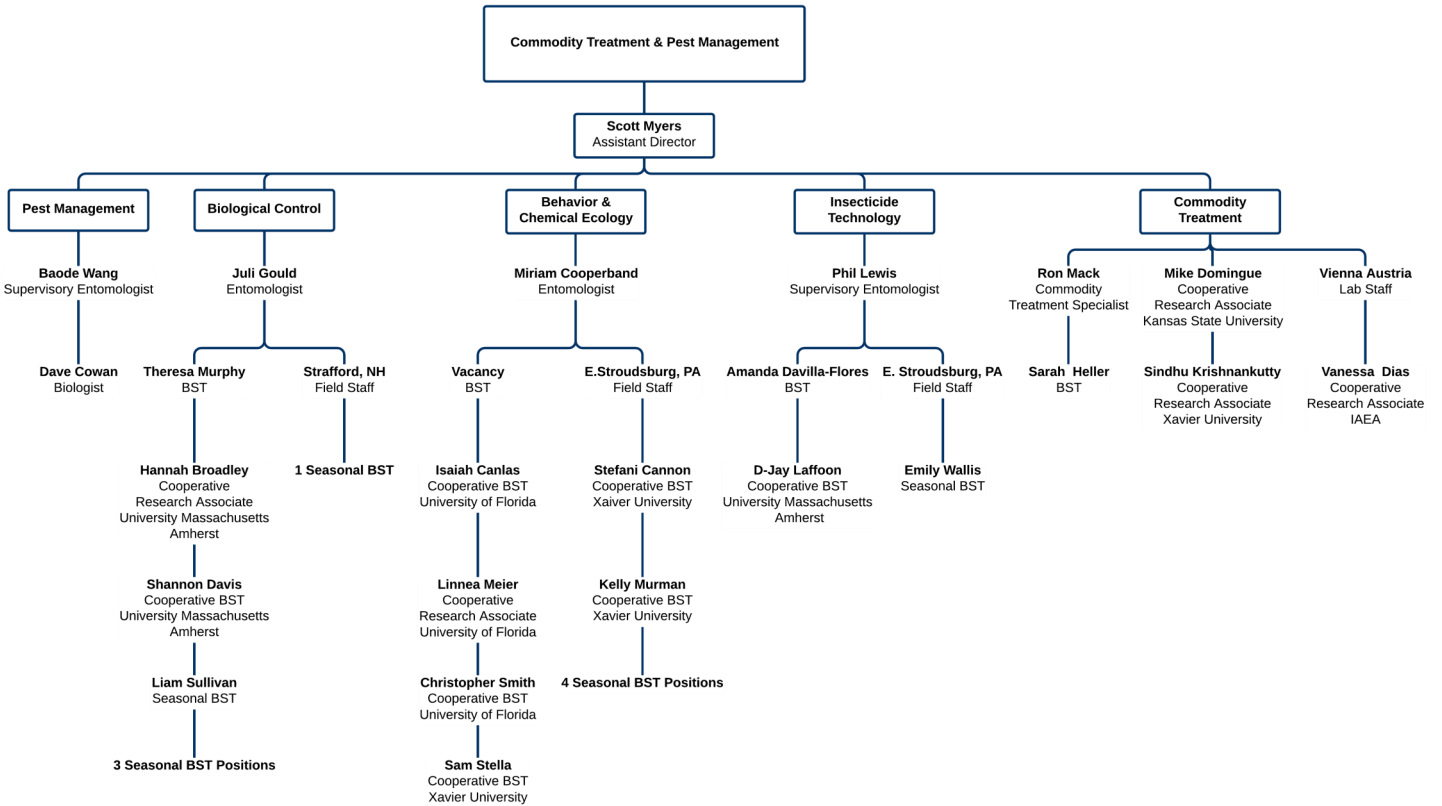
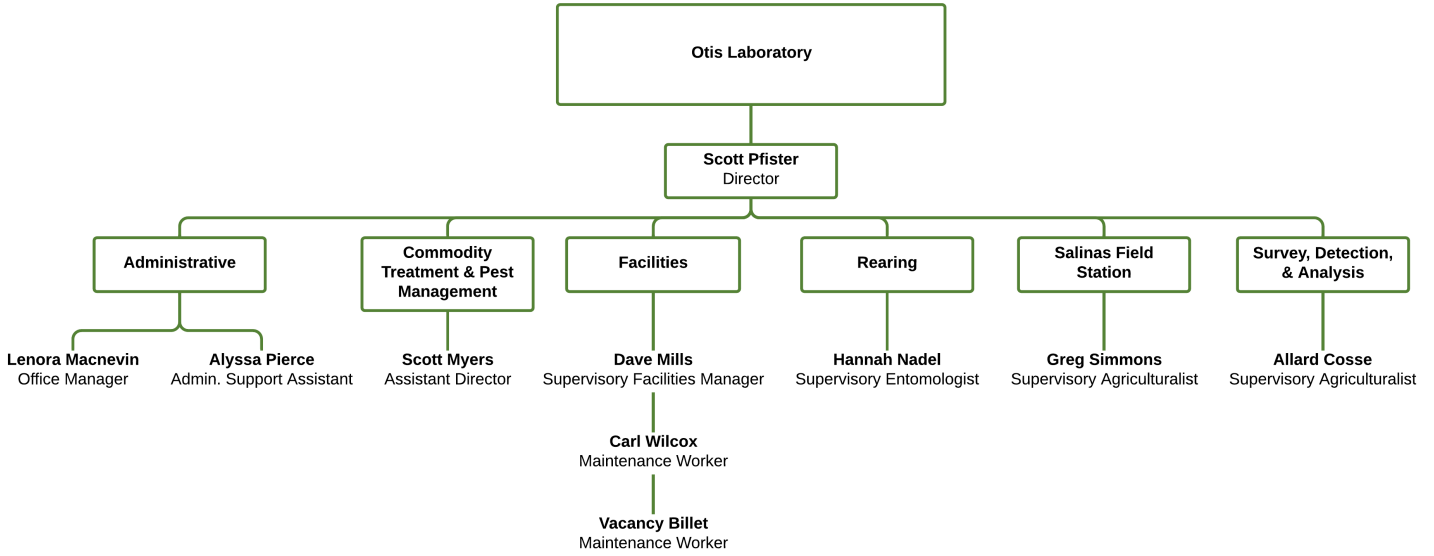
## Director's Message

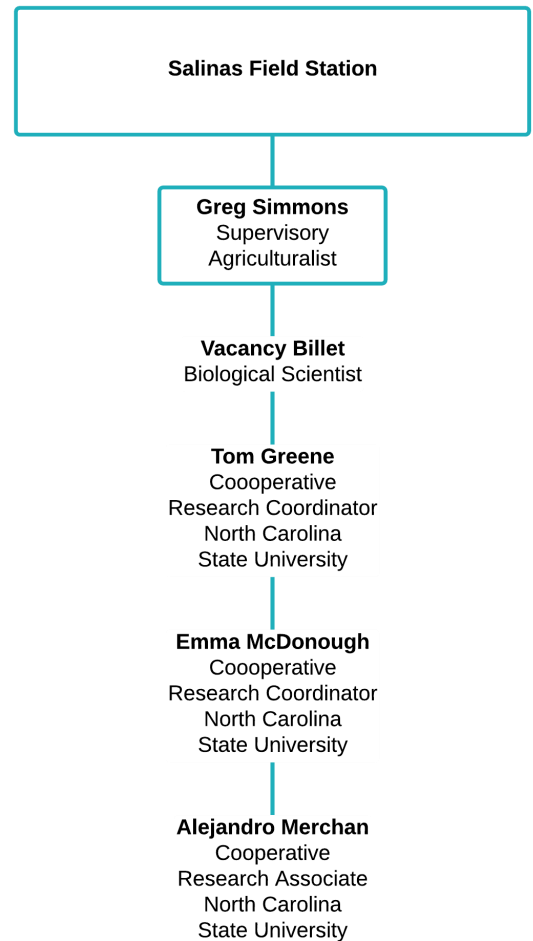
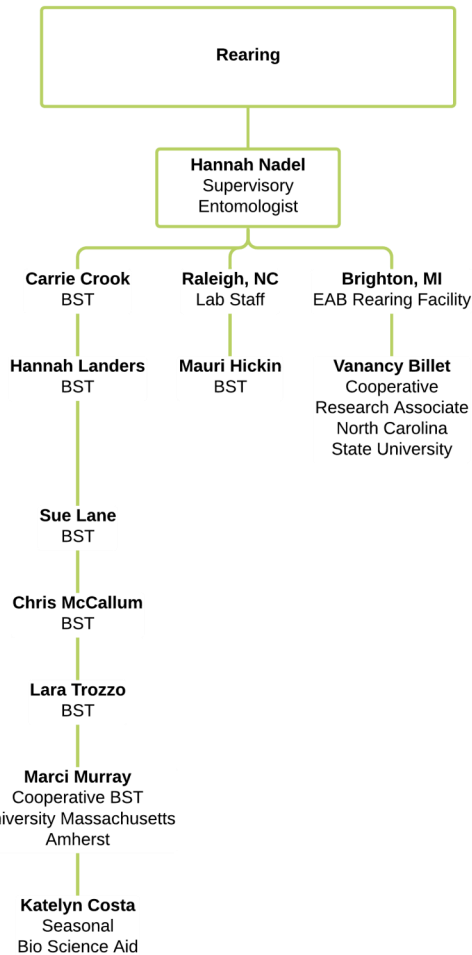
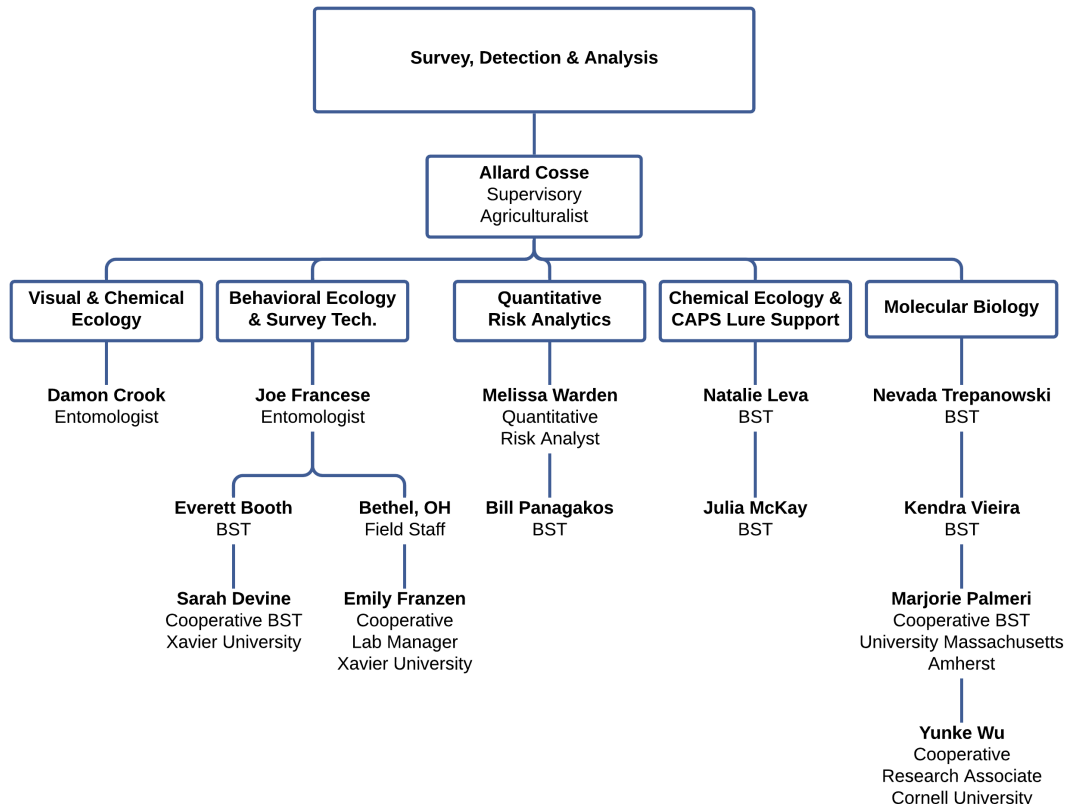
Scott Pfister

This 2018 Otis infographic relays a great deal of information about the laboratories accomplishments. However, the first thing I think about while reflecting on the graphic is that it speaks volumes about the quality of the employees affiliated with Otis. Collectively, the 68 federal, seasonal, and cooperative employees form a collaborative team that consistently produce quality results. Many attributes make up a successful team, but there are a few that I think exemplify Otis. First and foremost, there is a clear sense of purpose to the work, which in turn supports the larger organization. Commitment to achieving goals is the fuel which drives each member's efforts. Each Otis team member knows there is work to do, and they strive to get it done. Another defining aspect of Otis is the prioritization of team-centered communication, where employees carefully listen to each other, place value on their colleagues' input, and receive thoughtful feedback from their peers and supervisors. Finally, effective teams recognize that there are many improvement opportunities and, they are all about growing better together. Otis's understanding of the value of team growth mindset provides the foundation for a culture of continuous improvement where the team is willing to take risks, be creative, and not afraid of setbacks. I am very proud to be part of the Otis team.



Created by: Nevada Trepanowski







## Tolerance of four South American fruit fly, *Anastrepha fraterculus*, morphotypes to cold treatment

Vanessa S. Dias<sup>1,2</sup>, Amanda A. S. Cardoso<sup>1</sup>, Nick V. Hurtado<sup>1</sup>, Guy J. Hallman<sup>1</sup>,  
Carlos E. Caceres<sup>1</sup>, Marc J.B. Vreysen<sup>1</sup>, and Scott W. Myers<sup>2</sup>

<sup>1</sup>Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, IAEA, Vienna, Austria

<sup>2</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

### Introduction

Cold treatment is one of the most widely applied phytosanitary measures in the world. The development of generic cold treatments for fruit flies is desirable because it facilitates the treatment application and efficacy against different quarantine species. Broadly applicable cold treatment schedules, however, must consider potential differences in cold tolerance among various fruit fly species and their populations.

The development of a generic cold treatment against fruit flies is particularly challenging for cryptic species of the *Anastrepha fraterculus* complex (Figure 1). Several studies have shown that *A. fraterculus* populations can differ substantially at the molecular, cellular, morphological, and behavioral levels. In particular, morphometric studies have indicated that the *A. fraterculus* complex is currently comprised of at least eight morphotypes that might soon be described as several new species. These eight *A. fraterculus* morphotypes are named Andean, Brazilian-1, Brazilian-2, Brazilian-3, Ecuadorian, Mexican, Peruvian, and Venezuelan.

Considering that the nominal species *A. fraterculus* is indeed a complex of several cryptic species, it is essential to test whether the South American populations of the *A. fraterculus* complex differ in cold tolerance to anticipate the use of a generic treatment against all morphotypes, especially in view of any future species that may arise from them. This is particularly important considering that the few published studies evaluating cold tolerance of the nominal species *A. fraterculus* were focused on just two morphotypes, Brazilian-1 from Argentina and Andean from Colombia.

### Assessing cold tolerance of *A. fraterculus* morphotypes

The *A. fraterculus* morphotypes Brazilian-1 (Castelar and Tucumán, Argentina), Ecuadorian (Cusco, Peru), Andean (Ibagué, Colombia), and Peruvian (La Molina, Peru) were assessed to determine if they had significantly different cold tolerance. Experiments were carried out at the Insect Pest Control Laboratory of the Joint FAO/IAEA Agriculture & Biotechnology Laboratories using nectarines naturally infested with the four morphotypes. Infested nectarines were held until larvae reached the third instar, which is considered the most cold tolerant stage.



**Figure 1.** *Anastrepha fraterculus* (Brazilian-1 morphotype) on nectarine.



Nectarines infested with 3<sup>rd</sup> instar larvae were then placed into a cold chamber at  $1.37 \pm 0.1^\circ\text{C}$  for 8, 9, 10 and 15 days (Castelar, Cusco, Ibagué, La Molina) or at  $1.50 \pm 0.1^\circ\text{C}$  for 8, 9, 10, 15 and 17 days (Tucumán). Control groups were maintained at  $25^\circ\text{C}$  to estimate natural mortality. All nectarines were dissected within two days post-treatment. The number of pupae and third-instar larvae (live and dead) was recorded. Treatment efficacy was determined by acute larval mortality.

## Results

Few 3<sup>rd</sup> instar larvae from the Brazilian-1 and Ecuadorian morphotypes survived low temperatures at sub-lethal doses (8 and 9 days). Infested nectarines exposed to  $1.37^\circ\text{C}$  for 15 days yielded no survivors for all morphotypes (Table 1). However, nectarines infested by the Brazilian-1 morphotype from Tucumán exposed to  $1.50^\circ\text{C}$  for 15 days yielded one survivor (Table 1).

While this survivor was moving and thus counted as alive, it died as a coarctate larva and did not survive to the adult stage. Increasing the duration of cold treatment at  $1.50^\circ\text{C}$  for nectarines infested by the *A. fraterculus* population from Tucumán to 17 days yielded no survivors.

## Conclusion

These results suggest that the USDA PPQ treatment schedule for *Anastrepha* spp. other than *A. ludens* (T107-a-1, 15 days at  $1.11^\circ\text{C}$  or 17 days at  $1.67^\circ\text{C}$ ) has the potential to be used as a generic treatment for any future species that may arise from the *A. fraterculus* complex.

**Table 1.** Mean mortality ( $\pm$  SE) of four morphotypes of *A. fraterculus* complex larvae treated in nectarines at  $1.37$  or  $1.50 \pm 0.1^\circ\text{C}$ .

Population (morphotype)	Replicates (Total no. treated)	Time at $1.37$ or $1.50 \pm 0.1^\circ\text{C}$					
		0 days (control)	8 days	9 days	10 days	15 days	17 days
Castelar (Brazilian-1)	11 (26,758)	$3.96 \pm 1.51$	$96.16 \pm 2.57$	$99.77 \pm 0.15$	$100.00 \pm 0.00$	$100.00 \pm 0.00$	-
Cusco (Ecuadorian)	10 (22,548)	$8.27 \pm 3.00$	$99.98 \pm 0.02$	$100.00 \pm 0.00$	$100.00 \pm 0.00$	$100.00 \pm 0.00$	-
Ibagué (Andean)	11 (24,065)	$6.79 \pm 3.00$	$100.00 \pm 0.00$	$100.00 \pm 0.00$	$100.00 \pm 0.00$	$100.00 \pm 0.00$	-
La Molina (Peruvian)	10 (27,978)	$7.91 \pm 3.44$	$100.00 \pm 0.00$	$100.00 \pm 0.00$	$100.00 \pm 0.00$	$100.00 \pm 0.00$	-
Tucumán (Brazilian-1)	10 (14,647)	$4.72 \pm 1.86$	$94.79 \pm 2.55$	$99.15 \pm 0.00$	$100.00 \pm 0.00$	$99.98 \pm 0.02$	$100.00 \pm 0.00$

## Effect of low-oxygen conditioning on radiotolerance of Mediterranean fruit fly and oriental fruit fly

Vanessa S. Dias<sup>1,2</sup>, Nick V. Hurtado<sup>1</sup>, Amanda A. S. Cardoso<sup>1</sup>, Camilo Rivera<sup>1</sup>, Florence Maxwell<sup>1</sup>, Guy J. Hallman<sup>1</sup>, Carlos E. Caceres<sup>1</sup>, Marc J.B. Vreysen<sup>1</sup>, and Scott W. Myers<sup>2</sup>

<sup>1</sup>Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, IAEA, Vienna, Austria

<sup>2</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

### Introduction

Ionizing radiation has been successfully used as a phytosanitary treatment to disinfest commercial fruit and facilitate international trade by reducing quarantine barriers. To ensure the consistency and effectiveness of phytosanitary irradiation, multiple aspects that may affect its ability to prevent insect development or reproduction must be considered, such as the determination of target doses and organismal radiotolerance.

Prior studies focusing on the definition of target doses against fruit flies have significantly contributed to the use of phytosanitary irradiation worldwide. However, research is needed to evaluate whether modified atmosphere storage, a treatment widely applied to preserve commodity quality, has an impact on the efficacy of phytosanitary irradiation against tephritid fruit flies. Most studies on this topic have focused on the effect of either very low levels of oxygen or modified atmosphere packaging (MAP) on the efficacy of phytosanitary irradiation against a few fruit fly species. Thus, it is essential to use a consistent methodology in studies comparing multiple tephritid species to evaluate the extent to which insect conditioning at low to moderate oxygen levels before and during irradiation may affect their survival and development.

### Experimental Approach

In this study, the effect of low-oxygen conditioning on the efficacy of phytosanitary irradiation was evaluated simultaneously for oriental fruit fly, *Bactrocera dorsalis* and Mediterranean fruit fly, *Ceratitis capitata* using the same methodology and approach. The *B. dorsalis* colony (~F-70) was originally collected in wild-infested mangoes in Kenya. The *C. capitata* colony (~F-25) was obtained from wild-infested oranges in Argentina.

Experiments were done at the Insect Pest Control Laboratory of the FAO/ IAEA Agriculture and Biotechnology Laboratories, Seibersdorf, Austria, using naturally infested mandarins. Two mandarins infested with 3<sup>rd</sup> instar larvae of *B. dorsalis* or *C. capitata* were placed inside a plastic chamber (12.5 cm diameter × 35 cm high) that contained a screw lid on the top sealed with vacuum grease and two plastic luer-lock valves attached to the bottom and top sides for gas flushing. Three pieces (1 cm x 1 cm) of Gafchromic<sup>®</sup> films were positioned under and above each mandarin treated with ionizing radiation to measure the absorbed dose at different positions. Hypoxia (5-6% of O<sub>2</sub>) and severe-hypoxia (< 1% of O<sub>2</sub>) treatments were achieved by flushing the plastic chambers with gas mixtures containing argon, carbon dioxide, nitrogen, and oxygen at different concentrations. These gas mixtures simulated the possible mild to extreme atmospheric conditions during phytosanitary irradiation of controlled atmospheres, in which oxygen can be partially or completely replaced by carbon dioxide due to fruit respiration. Oxygen and carbon dioxide concentrations were monitored hourly using a Check-Mate 3 gas analyzer (Dansensor, Denmark). Infested mandarins were either conditioned under low-oxygen atmospheres for six hours or kept under ambient air before irradiation. Irradiation of infested mandarins with gamma rays covered a range of doses with sub-lethal and lethal effects for each fruit fly species.

After irradiation, each fruit was individually labeled and placed in a plastic container to allow insect development. Mandarins were dissected within seven days after treatment. The number of pupae and third-instar larvae (live and dead) was recorded. Treatment efficacy was determined by prevention of adult emergence.

## Results

Results suggest that hypoxic and severe-hypoxic conditioning before and during irradiation increases emergence of *B. dorsalis* (Table 1) and *C. capitata* (Table 2) at low doses of gamma radiation (>40 Gy). At higher irradiation doses, similar to those used in phytosanitary irradiation treatments, low-oxygen conditioning treatments did not increase the emergence rates of any fruit fly species evaluated.

## Conclusion

A similar study with two other economically important fruit fly species, *Anastrepha fraterculus* and *A. ludens* is planned for 2019. This research will make a significant contribution to the revision of restrictions applied by regulatory agencies to phytosanitary irradiation against fruit flies for commodities conditioned under low oxygen levels.

**Table 1.** Adult emergence (mean  $\pm$  SE) of *B. dorsalis* third instar larvae irradiated at different doses in normoxia, hypoxia, and severe hypoxia atmospheres.

Atmospheric conditions	Nominal irradiation dose (Gy)	Replicates	Total no. larvae treated	Adult emergence (%)
Normoxia (21% O <sub>2</sub> , 0% CO <sub>2</sub> )	0 (control)	36	19,400	84.00 $\pm$ 3.10
	30	10	1,495	4.00 $\pm$ 1.70
	40	16	4,863	2.00 $\pm$ 1.00
	80	11	2,701	0.01 $\pm$ 0.02
	116	52	6,671	0.00 $\pm$ 0.00
	150	18	6,433	0.00 $\pm$ 0.00
Hypoxia (5-6% O <sub>2</sub> , 15-16% CO <sub>2</sub> )	0 (control)	23	4,388	78.00 $\pm$ 5.30
	30	15	3,550	13.00 $\pm$ 4.74
	40	17	3,170	9.00 $\pm$ 3.86
	80	14	2,127	0.00 $\pm$ 0.00
	116	35	4,395	0.00 $\pm$ 0.00
	150	16	1,873	0.00 $\pm$ 0.00
Severe-hypoxia (<1% O <sub>2</sub> , 21% CO <sub>2</sub> )	0 (control)	48	11,300	79.00 $\pm$ 3.60
	30	10	2,197	43.00 $\pm$ 7.19
	40	16	3,841	18.00 $\pm$ 5.10
	80	9	1,427	0.00 $\pm$ 0.00
	116	37	4,567	0.00 $\pm$ 0.00
	150	17	4,023	0.00 $\pm$ 0.00

**Table 2.** Adult emergence (mean  $\pm$  SE) of *C. capitata* third instar larvae irradiated at different doses in normoxia, hypoxia, and severe hypoxia atmospheres.

Atmospheric conditions	Nominal irradiation dose (Gy)	Replicates	Total no. larvae treated	Adult emergence (%)
<b>Normoxia</b> (21% O <sub>2</sub> , 0% CO <sub>2</sub> )	0 (control)	23	6,614	85.00 $\pm$ 3.34
	20	2	69	15.00 $\pm$ 12.70
	30	9	2,495	3.00 $\pm$ 2.16
	50	5	351	0.00 $\pm$ 0.00
	70	4	369	0.00 $\pm$ 0.00
	100	21	3,091	0.00 $\pm$ 0.00
<b>Hypoxia</b> (5-6% O <sub>2</sub> , 15-16% CO <sub>2</sub> )	0 (control)	8	931	79.00 $\pm$ 3.39
	20	4	277	24.00 $\pm$ 7.10
	30	7	446	7.00 $\pm$ 4.68
	50	6	453	0.00 $\pm$ 0.00
	70	5	977	0.00 $\pm$ 0.00
	100	18	1,551	0.00 $\pm$ 0.00
<b>Severe-hypoxia</b> (<1% O <sub>2</sub> , 21% CO <sub>2</sub> )	0 (control)	9	3,622	76.00 $\pm$ 6.70
	20	6	409	60.00 $\pm$ 8.65
	30	7	1,113	15.00 $\pm$ 7.54
	50	6	233	0.00 $\pm$ 0.00
	70	10	1,424	0.00 $\pm$ 0.00
	100	12	1,551	0.00 $\pm$ 0.00

## Ethanedinitrile as a methyl bromide alternative for pine log exports

Scott W. Myers<sup>1</sup>, Amanda Davila-Flores<sup>1</sup>, and Kendhl Seabright<sup>2</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>University of Tennessee, Institute of Agriculture, Knoxville, TN

### Introduction

The movement of invasive pests through the international trade of wood products is a serious concern. Many species have been introduced through this pathway, resulting in devastating effects to native forests and forestry industries. Phytosanitary treatments are often used to mitigate the risk of introduction of these pests.

In the U.S. whole log exports present a unique challenge for phytosanitary treatment as they are typically shipped shortly after harvest with bark intact. This means there is the potential for logs to harbor a number of pests including wood boring insects and wood pathogens. Among the greatest concern for North American log exports is the pinewood nematode, PWN, *Bursaphelenchus xylophilus*. This pest is regarded as the most important pest for development of soft wood treatments in international trade.

The fumigation of logs for export from the U.S. represents one of the largest quarantine and pre-shipment use exemptions for methyl bromide, an ozone depleting compound that has been subject to global phase out. Log fumigations require high rates of methyl bromide to kill pinewood nematodes and there are no commercially available alternative treatments. The development and adoption of efficacious and economically viable alternative fumigants would facilitate compliance with state and federal air quality restrictions, which limits its use. Additionally alternative fumigants have the potential to benefit industry, and support the efforts of domestic and international regulatory agencies to facilitate international trade.

Ethanedinitrile (EDN), a newly registered fumigant in New Zealand and Australia, has shown potential as an alternative to methyl bromide fumigation for the treatment of logs to control various invasive pests.

The objective of this study was to investigate the efficacy of EDN in controlling PWN in pine wood. We conducted a series of experiments using 10 cm pine blocks followed by 1.2 m log sections to simulate the conditions of a commercial fumigation treatment.

### Methods

To simulate logs, 10 cm<sup>3</sup> blocks of *Pinus virginia* with bark intact were first inoculated with fungi to provide a food source for PWN. Next, inoculum of PWN was added and the blocks were incubated for 4-6 weeks. Blocks were then sealed with paraffin on all surfaces except for the bark face. This method required through-bark penetration of the fumigant to reach PWN in the wood, as would be the case in a commercial log treatment.

Follow up experiments used a similar fungi and PWN inoculation method with freshly harvested white pine trees that were cut into 1.2 m log sections prior to treatment. Logs were inoculated with PWN stock solution at six locations in each log. Logs were then end sealed with paraffin to reduce drying and evaluations of nematodes in each log were made pre- and post-treatment to evaluate EDN treatment efficacy.

Blocks were fumigated in 10 L glass jars and logs were fumigated in 664 L stainless steel chambers, each for 24 hours at 20°C. Both types of enclosures were equipped with tube fittings and septa to enable gas sampling from the headspace. Wood samples were acclimated to the treatment temperature 24 hours prior to fumigation and were randomly assigned to a treatment or control group.

Fumigant doses were calculated volumetrically based on the volume of the empty chambers and using the ideal gas law to correct for atmospheric pressure and temperature. Headspace samples were measured at four hour intervals using a micro gas chromatograph throughout the treatments. After treatment, samples were individually bagged for post-treatment recovery assessment for the presence of surviving PWN. Recovery assessment for wood blocks and logs were evaluated before treatment and 21 days after treatment to provide a comparison of treatment efficacy. PWN were extracted from subsamples of each block and log using the Baermann funnel technique to estimate the number of nematodes per gram.

**Results**

Pine blocks were fumigated with treatments of 40, 60, or 80 mgL<sup>-1</sup> EDN for 24 hours with each replicated four times. All EDN concentrations effectively eliminated pinewood nematodes from all of the treated blocks (Table 1). Two blocks were untreated and used as controls to compare treatment efficacy using EDN and to determine natural mortality of PWN over the duration of treatment and post-treatment evaluation.

Sixty two pine logs were treated in five replicated treatments of 0, 40, 60, or 100 g/m<sup>3</sup> EDN for 24 hours. Again, there were no surviving nematodes in any of the treated logs at the 21 day post-treatment assessment (Table 1). Twenty two pine logs were used as untreated controls, with ten of them possessing no PWN before treatment, indicating some of the material was not well infested. However, only two controls had no PWN before and after treatment. This result suggests that the delay we experienced inoculating logs with fungi and PWN resulted in wood moisture content loss, which led to reduced PWN numbers in the wood. Nonetheless, both treatments provided quarantine level control and support continued work with EDN as a methyl bromide replacement for this purpose.

**Conclusion**

Future research with EDN as a log treatment should explore lower temperature and shorter duration treatments to optimize treatment parameters for industry. Additionally, hardwood log exports require treatment for the oak wilt pathogen which is another potential use for EDN.

The development of EDN as an alternative for methyl bromide would enable treatment and export of logs with little disruption to current practices. While EDN has demonstrated potential as a replacement for methyl bromide, it is not yet registered for use in the U.S. Assuming the registration process goes forward, existing fumigation facilities could be used to efficiently treat valuable logs, ensuring compliance with international phytosanitary standards and preserving product quality. Additionally, broadening our efforts to find suitable replacements for methyl bromide will enhance our role as a global leader in safe, efficacious, and environmentally sound treatments.

**Table 1.** Ethanedinitrile treatment of pinewood nematode infected *Pinus virginiana* blocks and *P. strobus* logs for 24 hours at 20°C.

Treatment type	Concentration (mgL <sup>-1</sup> )	Pre-treatment		Post-treatment (21 days)	
		Mean no. PWN/g	No. with PWN/Total no.	Mean no. PWN/g	No. PWN/Total no.
10 cm <sup>3</sup> blocks	Control	751	2/2	15.7	2/2
	40	363	8/8	0.00	0/8
	60	331	8/8	0.00	0/8
	80	1,200	7/7	0.00	0/7
1.2 m logs	Control	2.0	12/22	1.9	19/22
	40	1.0	15/20	0.0	0/20
	60	1.8	12/22	0.0	0/22
	100	1.9	13/20	0.0	0/20



## Preference of khapra beetle for attractants in competitively placed traps

Michael J. Domingue<sup>1,2</sup>, Scott W. Myers<sup>1</sup>, and Christos G. Athanassiou<sup>3</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>Kansas State University, Department of Entomology, Manhattan, KS

<sup>3</sup>University of Thessaly, Department of Agriculture Crop Production and Rural Environment, Nea Ionia, Magnesia, Greece

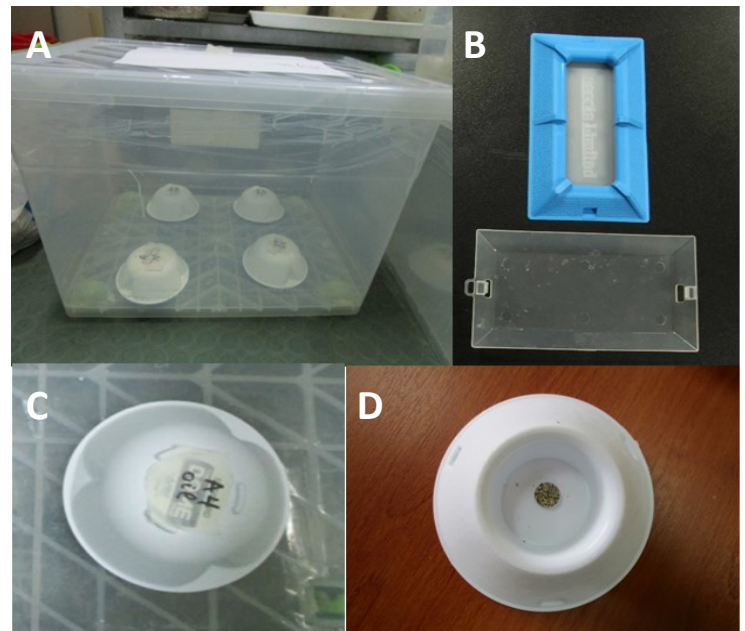
### Introduction

Khapra beetle, *Trogoderma granarium*, is a major pest that infests a variety of stored products and dried foods and is an important quarantine insect species in many countries. *Trogoderma granarium* is native to India, but has been found in different parts of the world including the United States, Europe (e.g., Germany and United Kingdom), South Asia, and Africa [1,2,3]. Khapra beetle is recognized as one of the 100 most invasive species globally [4]. The optimum temperature for its development is 35°C. The key environmental conditions for the quick development of this species is low relative humidity and high temperature [2]. At temperatures below 25°C larvae can go into diapause. Diapause of larvae is the most important characteristic of *T. granarium* biology because it increases their ability to survive long periods and to resist control measures.

Most of the studies surrounding khapra beetle are focused on the evaluation of different semiochemicals and traps, however both are continuously renewed in the market. Therefore, it is essential to evaluate the available trapping devices as well as the attractants that are commercialized to identify potential improvements in monitoring protocols. Recently it was determined in a series of laboratory bioassays that the PantryPatrol gel (Insects Limited, Inc.), was the most effective lure for eliciting upwind movement, and arrestment in larvae [5]. In the present study, we evaluated the two traps we refer to as dome trap (Trece, Inc.) and box trap (Insect Limited, Inc.) with competing attractants for adults and larvae; only the results obtained for adults are presented.

### Methods

*Trogoderma granarium* were reared at the Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly in whole wheat kernels, at 34°C, 65% RH, and continuous darkness. For all species, adult beetles less than one month-old were used in the following tests. Trials were conducted inside plastic boxes that were placed in incubator chambers set at the above conditions (Figure 1). In the middle of each box, 30 adults of *T. granarium* were released and kept covered to be acclimated for five minutes.



**Figure 1.** A) Behavioral assay arena, which had four trap choices in the different corners. In each trial either B) box traps (Insect Limited, Inc.) or C) dome traps (Trece) were used. D) Attractants were placed directly in traps as shown for the Dermestid tablet (in a dome trap).

Within each of the boxes four separate traps were placed in the corners, all either dome or all box traps in a given trial. Different odors were used within the traps in each of the trials. Thus it could be evaluated which odors were most successful at competitively eliciting positive responses leading to trap captures. Different traps were never used within the same arena, so the competitive effect of one trap type over the other was not assessed.

We were interested in four odors to evaluate alongside a control with no odor. Thus the five treatments (attractants) were evaluated:

- 1) 0.13 g of PantryPatrol gel (Gel)
- 2) 0.13 g of oil-based kairomone food attractant (Oil) (Storgard Oil, Trece, Inc.)
- 3) 0.13 g of wheat germ (Wheat Germ) (Honeyville, UT, USA)
- 4) 0.13 g of Dermestid tablet attractant (Tab) (Insects Limited, Inc.)
- 5) Control without any attractant

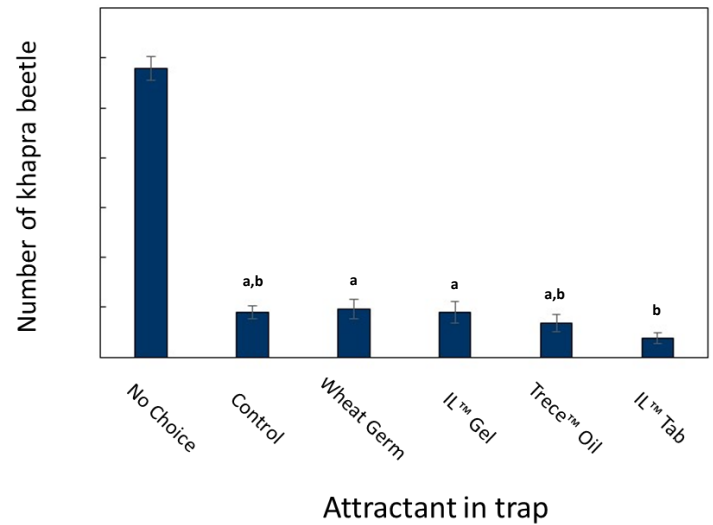
Because the design only allowed the comparison of four treatments at a time; the following combinations with four odor choices per arena were each tested three times. The protocol was repeated separately for both of the two trap types:

- 1) Wheat Germ, Oil, Control, Tab (no Gel)
- 2) Gel, Tab, Wheat Germ, Control (no Oil)
- 3) Wheat Germ, Oil, Gel, Control (no Tab)
- 4) Oil, Tab, Gel, Control (no Wheat Germ)
- 5) Wheat Germ, Tab, Oil, Gel (no Control)

After seven days, the boxes were opened and the adults inside each trap/attractant combination were counted. The number of dead and live adults outside of the traps (no choice adults) for each trial were counted.

## Results

Data were analyzed by examining the mean number of insects trapped in a given lure and trap type combination across all trials of the experiment. In each case, almost two thirds of the adults did not enter any of the four traps within the arena (Figure 2). An analysis of variance showed only marginal statistical effects of trap type (1 d.f.  $F = 3.36$ ,  $p = 0.078$ ) and odor (4 d.f.,  $F = 2.65$ ,  $p = 0.056$ ). The interaction was not significant (4 d.f.,  $F = 2.14$ ,  $p = 0.105$ ). Grouping the treatments across both trap types as we have done here, shows that there was generally little effect of having an odor in the trap for increasing captures at this close range (Figure 2). The control traps had mean captures similar to traps with odors. In fact, the lowest trap captures were in the traps with Tab, which had a capture rate marginally less than the wheat germ and Gel treatments.



**Figure 2.** Mean  $\pm$  SE for number of larvae in each behavioral category across all trials of the experiment, combining the use of dome and box traps. An analysis of variance with a comparison of means using a Tukey correction was performed for the number of beetles found in each trap. Differing letters on the figures show that the means were marginally significantly different ( $p < 0.1$ ).

## Conclusion

Further research will continue to determine if a similar effect is seen in larvae with regards to the competitive ability of lures in closely situated traps. The Gel bait was previously found to be highly attractive as a standalone odor source to larvae [5]. It may be possible that this lure is not as effective when placed in areas where there are likely to be competing odors.

## References

- 1) Zacher F., 1938. Verschleppung und einbürgerung von vorratsschlingen. In VII. Internationaler Kongress für Entomologie. Berlin: Friedrich-Wilhelms-Univ. 2919–2926.
- 2) Banks H.J., 1977. Distribution and establishment of *Trogoderma granarium* Everts (Coleoptera: Dermestidae): climatic and other influences. J. Stored Prod. Res. 13:183–202.
- 3) Athanassiou C.G., Phillips T.W., and Wakil W., 2019. Biology and Control of the Khapra Beetle, *Trogoderma granarium*, a Major Quarantine Threat to Global Food Security. Annual Review of Entomology 64:131–148.
- 4) Lowe S., Brone M., Boudjelas S., and De Poorter M., 2000. 100 of the World's Worst Invasive Alien Species. A Selection from the Global Invasive Species Database. Hollands Printing Ltd, Auckland.
- 5) Morrison, W.R., Grosdidier R., Arthur F., Scott Myers. S., and Domingue, M.J., Attraction, arrestment, and preference by immature *Trogoderma variabile* and *Trogoderma granarium* (Coleoptera: Dermestidae) to commercially available lures. Journal of Pest Science, In Press.



## Solid wood packing material destruction

Sarah Heller<sup>1</sup> and Ron Mack<sup>1</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

### Introduction

The objective of this study was to find a mechanical destruction method that could handle large quantities of solid wood packing material (SWPM) and destroy a variety of pests. The impetus for this study came from the port of Houston's concern over large quantities of abandoned SWPM, since the origin of the material, how it was treated, and what pests it might harbor is unknown. Because of air quality restrictions, the abandoned SWPM cannot be burned, so an alternative destruction method was required. Schutte Buffalo, a manufacturer of size-reduction equipment, makes a machine that can handle the size of dunnage and pallets, and can reduce wood to a fine powder (Figure 1). It is a two-step process, where the first piece of machinery grinds wood product, which is subsequently processed in a hammermill that can be adjusted to produce an end-product ranging from grindings to wood flour, depending on the screen size. Experiments were conducted to characterize the equipment's ability to handle material and destroy insect pests. We focused on cerambycids and scolytids, as those were pests of concern for the port of Houston and represent variation in pest size.

### Methods

#### *Cerambycid Experiments*

Pine pallets and dunnage were artificially seeded with Superworm larvae, *Zophobas morio*, a cerambycid surrogate, and then glued shut with a painted cork plug. All pallets and dunnage were processed through the grinder with a two inch screen. Each pallet was processed individually, while dunnage was processed in groups of three. Material was collected from the grinder and examined for surviving larvae. This experiment was repeated using red oak dunnage to observe the machine's ability to process hardwood.

Remaining ground pine and oak material were processed through the hammermill at varying screen sizes to understand processing ability and output material size. Five gallons of material were processed at a time through screens ranging from 1/32 inch to 3/8 inch screen.

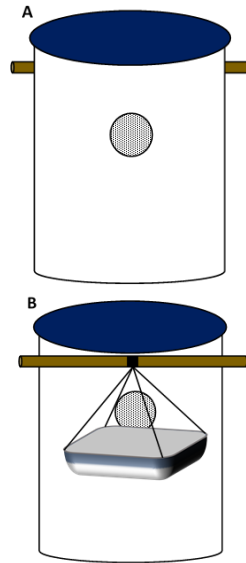
#### *Scolytid Experiments*

Red, white, and Scots pine trees infested with scolytid bark beetles were cut down from Shawme Crowell State Forest in Sandwich, MA. Trees were cut into three-foot sections, then quartered with the bark on. One quarter from each three-foot section was set aside as a control log, and the remaining three logs from each section were treated together.



**Figure 1.** Equipment tested for the destruction of abandoned dunnage and pallets at the port of Houston, manufactured by Schutte-Buffero.

Tree sections were transported to Schutte-Buffalo for treatment. Half of the replications were processed through the grinder (two inch screen), and the other half were processed through both the grinder (two inch screen) and the hammermill (3/8 inch screen). Samples of the processed material were collected and suspended in an emergence chamber. Emergence chambers consisted of a mesh-bottomed container, suspended in a 5-gallon food grade bucket (Figure 2). The mesh-bottomed container allowed surviving bark beetles to crawl out of the material and into the bottom on the five-gallon bucket. For replications that were processed through the grinder only, samples were taken at random and combined in the emergence chambers. For replications processed through the grinder and hammermill, a 2.5 gallon sample of the material from the grinder was processed through the hammermill. Then, samples were taken at random and combined in the emergence chambers. All emergence chambers were driven back to the Otis Laboratory and maintained in a laboratory setting at about 75°F. Beetles in the bottom of the emergence chambers were collected and counted after six weeks.



**Figure 2.** A) An external and B) internal view of the emergence chamber.

**Results**

Eight groups of pine dunnage and six pine pallets were processed through the grinder. In total, nine live Superworm larvae were found in the ground material (5/168 total insects for dunnage, 4/108 total insects for pallets).

Eight groups of red oak dunnage were processed through the grinder. Three surviving Superworm larvae were found in two different groups of ground material (3/168 total insects).

The grinder and hammermill were able to process the hardwood samples, but there appeared to be more variation in processing time and output material from the grinder when compared to softwood processing. Using the 3/8 inch screen, the hammermill produced material that was small enough to ensure insect destruction, while still allowing the machine to process efficiently. The smaller screen sizes produced smaller end product, but required more time.

No bark beetles were recovered in any of the red or white pine control groups, so it was determined that these trees did not contain the bark beetle infestation levels needed for this experiment. A total of eight beetles were recovered from three sets of Scots pine samples processed through the grinder (Table 1). No beetles were recovered from the hammermill samples.

**Conclusions**

The combination of the grinder and hammermill appears to be sufficient for the destruction of a wide range of insect pests in SWPM. The machinery is capable of handling hard and soft wood, dunnage, and pallets. The combined method would provide Houston with a mechanical destruction method for the SWPM abandoned at their port. We are currently working with Schutte Buffalo to develop plans for a grinder and hammermill set-up that can process the large amounts of SWPM left at ports. Engineers are aiming to design the machinery in the footprint of a shipping container, which would allow it to be portable within the infrastructure available at ports across the U.S.

**Table 1.** Beetles recovered from Scots pine trees treated with a grinder and hammermill.

	Replicate	Beetles recovered from controls	Beetles recovered from treated material
Grinder	2	0	1
	4	7	2
	6	4	0
	8	9	5
	10	15	0
	12	23	0
	14	11	0
Grinder and Hammermill	1	4	0
	3	0	0
	5	2	0
	7	4	0
	9	11	0
	11	7	0
	13	6	0

## Woods used as packaging for traded commodities and risk of wood-borer introduction through the wood packaging pathway

Hannah Nadel<sup>1</sup>, Sindhu Krishnankutty<sup>1,2</sup>, Yunke Wu<sup>1,3</sup>, Nevada Trepanowski<sup>1</sup>, Kendra Vieira<sup>1</sup>, Adam Taylor<sup>4</sup>, Michael Wiemann<sup>5</sup>, Steven W. Lingafelter<sup>6</sup>, Scott W. Myers<sup>1</sup>, and Ann M. Ray<sup>2</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>Xavier University, Department of Biology, Cincinnati, OH

<sup>3</sup>Cornell University, Department of Ecology and Evolutionary Biology, Ithaca, NY

<sup>4</sup>University of Tennessee, Tennessee Forest Products Center, Knoxville, TN

<sup>5</sup>Forest Products Laboratory, USDA USFS, Madison, WI

<sup>6</sup>USDA APHIS PPQ FO, Douglas, AZ

### Introduction

Despite international regulations aimed at preventing cargo from transporting pests in solid-wood pallets, crates, and other wood-packaging materials (WPM), non-native wood-boring insects are still discovered alive in WPM at United States ports of entry. Wood borers damage mostly woody plants by tunneling and feeding under the bark or inside the wood. They lay eggs in the bark of living or dying trees and shrubs, while some lay eggs in dry wood without bark. Their larvae usually tunnel into the wood to feed, and they develop into adult beetles after a year or more. When infested wood is turned into WPM, larvae can remain inside and may or may not show external evidence (tunnels, holes, waste materials) that they are present. Non-native insects in general are especially likely to become problems if they arrive in new areas because their predators and other natural enemies usually do not arrive with them. To kill insects inside WPM, international regulations stipulate that wood packaging be heated or fumigated before export; however, not all treatment is properly applied and some WPM manufacturers and commodity exporters ignore regulations and fail to treat their WPM. In some cases, wood may become infested after treatment if stored in the open. The larva, hidden inside the wood, is the most likely stage in the lifecycle to be transported with trade, while the adult insect tunnels out of the wood and is less often found with cargo.

Both hardwoods and softwoods are used in the construction of WPM for international trade; however, it is not clear if some types of wood pose higher risks than others for harboring wood-boring insects. This study revealed which types of trees were used to make the WPM that were inspected at ports and found to contain live wood boring insects. Less than 2% of cargo is inspected due to the high volume of trade arriving at ports, but the Department of Homeland Security's Customs and Border Protection (CBP) uses algorithms to select which WPM will be inspected by its agriculture specialists. Selections are based on the shipment's origin, the commodity, and whether there is a history of WPM infestation from the exporting company. Wood infested with wood borers is generally rejected and returned with the commodity to the exporter, or exchanged at the port and destroyed. During the study, CBP agriculture specialists collected live wood borers and samples of the tunneled wood during their inspections, and APHIS pest-identifiers sent them to Otis Laboratory for identification and research.

The study was conducted between April, 2012 and January, 2018 through a collaborative project between CBP and APHIS with the aim of identifying the live longhorned wood-boring beetles (family Cerambycidae) and jewel beetles (family Buprestidae) discovered at U.S. ports during WPM inspections. In 2016 the project was expanded to include wood wasps (family Siricidae). We received many, but not all, samples of infested wood packaging in addition to insects from ports, and identified the types of trees from which wood packaging was constructed. Collection of insect and wood specimens initially involved CBP and APHIS participants from six U.S. ports encompassing land, sea, and air arrivals, and expanded to 11 ports by 2016. Live insect specimens were shipped to the containment facility at the Otis Laboratory for rearing to the adult stage, followed by genus- or species-level identification using visual and/or molecular methods [1]. Wood was identified through visual characteristics by scientists at the University of Tennessee and the USFS Forest Products Laboratory in Madison, Wisconsin. Data on origins and types of traded commodities associated with the wood packaging were compiled from PPQ pest interception forms, diagnostic request forms, and from the PPQ Emergency Action Notification system database (EAN).

During the study we aimed to identify many of the wood-borers transported alive within WPM and to reveal the relationship between them and packaging woods, commodity types, and shipment origins. We also aimed to reveal whether the WPM pathway may have been responsible for the arrival of several destructive, non-native wood-borer species that are already present in the U.S. The identity of woods intercepted with live wood borers at ports is presented for consideration as a new factor for analyzing risk of pest entry posed by WPM. This may be useful towards refining algorithms used to select WPM for inspection, and for development of preemptive measures against non-native wood-boring insects that could enter the country.

### Results

We classified 486 wood-packaging samples as hardwoods or softwoods, and identified 480 samples to tree genus (Table 1). The infested WPM was constructed mostly of softwoods (78.8%). Five softwood genera belonging to two plant families and 29 hardwood genera belonging to 17 families were recognized.

Among the identified wood genera, the most frequently intercepted with live wood borers were pines, *Pinus* species (263 interceptions, 55%), spruces, *Picea* species (93 interceptions, 19%), and poplars, *Populus* species (48 interceptions, 10%). The wood packaging arrived at ports with consignments originating from 42 countries. Softwoods originated nearly equally from Asia (32.6%), Europe (34.8%), and North America (Mexico) (31.0%), while hardwoods originated predominantly from Asia (84.8%). The majority of WPM interceptions containing live wood borers were from Mexico, China, and Turkey; these countries contributed 34.2%, 12.8%, and 10.7%, respectively, of the interceptions for which we were able to identify both the infested wood and the wood borers.

The form of infested wood-packaging was sometimes recorded in the EAN as crating, pallets, and dunnage (fillers used to prevent items from shifting during transport), although more than half was recorded only as unspecified WPM (Table 2). Pallets were constructed mostly of softwoods (86%), mainly pines and spruces. Crating was constructed of both hardwoods and softwoods, and dunnage was made of softwoods, but too few of these packaging forms were recorded to allow meaningful conclusions. Four broad commodity categories were associated with the majority (82%) of infested WPM: 1) Stone, Ceramics, and Terracotta; 2) Vehicles and Vehicle Parts; 3) Machinery, Tools, and Hardware; and 4) Metal (Table 2). These constituted heavy commodities, whose WPM have been known to carry higher risk of wood borer infestation than WPM of lighter commodities. We found that, indeed, infested woods had a greater association with these commodities than with other commodities. Of 378 interceptions of softwoods linked with commodity data, 80% were associated with these four commodity categories; 86% of 102 hardwood interceptions were associated with them.

In pines we succeeded in classifying 24 species of longhorned and jewel beetles, at least seven more classified only to genus, and 135 specimens of longhorned and jewel beetles identified to family level only. In spruces we classified 20 species, at least eight more classified only to genus, and 50 unidentified longhorned beetles, jewel beetles, and wood wasps. Poplars contained 10 species, two more identified to genus only, and 13 unidentified longhorned beetles.

The majority of intercepted wood borers we succeeded in identifying to species were reported as pests in at least some part of their native or invaded ranges. Several have the capacity to spread plant diseases. None of the 18 identified wood borer species intercepted in hardwoods are native to the U.S., but three were introduced to the country before the study and now breed in parts of the country: Asian longhorned beetle, ALB, *Anoplophora glabripennis*, velvet longhorned beetle, VLB, *Trichoferus campestris*, and the yellow eucalyptus longhorned borer, *Phoracantha recurva*. These three were intercepted multiple times during the study (Table 3) and are therefore likely to have entered the country through the WPM pathway.

Among non-native species that attack softwoods, four have breeding populations in the U.S., including VLB (which attacks softwoods and hardwoods); a violet-colored longhorned beetle, *Callidium violaceum*; *Arhopalus rusticus*; and the old-house borer, *Hylotrupes bajulus*. They are all economically significant pests of dry wood, while VLB may damage live fruit trees such as cherry and peach. Six species native to the U.S. that were transported in softwoods from Mexico are considered pests in areas outside of their home regions. At least 17 of the wood borer species develop in living and lightly stressed trees and therefore pose a threat to standing trees in forests, urban landscapes, and agriculture, but many of them also develop in declining and dead trees and pose a threat to timber and wooden structures. About 10 more species appear to prefer dead to dry or decaying wood, and could become timber pests if they start breeding in the country.

A notable finding from this study was the absence of ash, *Fraxinus* species, among the intercepted packaging woods. This suggests that WPM may not have been the pathway of entry for the emerald ash borer, EAB, *Agrilus planipennis*, into North America. This east-Asian jewel beetle, which feeds nearly exclusively under the bark of ash, has been devastating natural ash forests and plantings on the continent and was presumed to have arrived in WPM with attached bark. Only eight interceptions of EAB are recorded in the APHIS PestID interceptions database and none are linked to WPM. All occurred in land borders between the U.S. and Canada after the pest was already present in both countries.

## Conclusions

This study revealed for the first time the diversity of packaging woods in which live wood borers are transported to the U.S. through global trade, and the identities of many of the wood-borer species carried within them. It provides the identities of infested wood genera commonly used to construct WPM for trade goods and that harbored live wood borers during inspection by CBP. The three most frequently intercepted wood genera were revealed along with the countries from which the consignments originated. Wood packaging was confirmed as a pathway of entry for several non-native wood-boring pests that invaded the U.S. in the past and are present in breeding populations, but is debatable as a pathway for entry by EAB. Data on packaging-wood types and the pests they carry can be used to assess threat posed by infested WPM and provides advanced warning of several non-native forest, agricultural, and structural pests at risk of entering the country.

## Supplementary Tables

- 1) Nadel et al. Tables 1-3. Otis SharePoint Data Archive. [2018 Annual Report Data](#).

## References

- 1) Wu Y, Trepanowski N, Molongoski J, Reagel P, Lingafelter S, Nadel H et al. Identification of wood-boring beetles (Cerambycidae and Buprestidae) intercepted in trade-associated solid wood packaging material using DNA barcoding and morphology. *Scientific Reports*. 2017;7: 40316.



## Development of biological control methods against spotted lanternfly

Hannah J. Broadley<sup>1,3</sup>, Juli R. Gould<sup>1</sup>, Kim A. Hoelmer<sup>2</sup>, and Xiao-yi Wang<sup>4</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>Beneficial Insects Introduction Research Unit, USDA ARS, Newark, DE

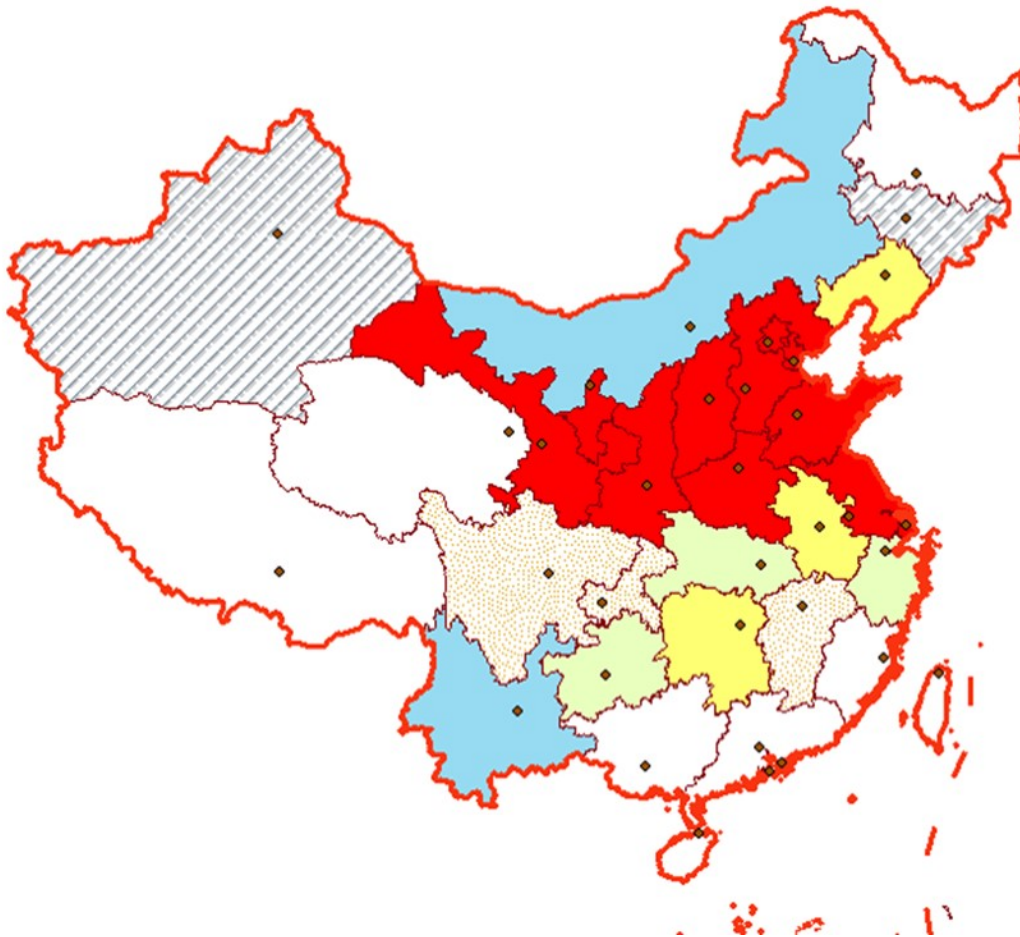
<sup>3</sup>University of Massachusetts, Department of Environmental Conservation, Amherst, MA

<sup>4</sup>Chinese Academy of Forestry, Research Institute of Forest Ecology, Environment and Protection, Beijing, China

### Introduction

The spotted lanternfly, SLF, *Lycorma delicatula*, was discovered in Berks County Pennsylvania in 2014 and has since spread to 12 additional counties in Pennsylvania. Additionally, SLF has established in Delaware, New Jersey, and Virginia and has been detected in Maryland, Massachusetts, and New York. Unfortunately, populations of spotted lanternfly are spreading fast. Given the eradication challenges, we are working to develop a biological control program against the spotted lanternfly. Potential classical biological control agents were identified through publications and previous work from South Korea and China.

SLF is native to China and is present in 20 provinces (Figure 1), where it is generally not considered a pest. Some pests, such as emerald ash borer, are suppressed by host plant resistance. However, SLF's preferred host tree (tree-of-heaven, *Ailanthus altissima*) is the same in Asia and North America, suggesting that natural enemies are maintaining SLF populations at low densities in China. SLF was first detected in South Korea in 2004 and is considered a pest there; populations are also widespread in Japan.



**Figure 1.** Provinces in China with known spotted lanternfly populations. Provinces colored in red, green, and yellow show areas with relatively high, medium, and low densities of SLF, respectively. Blue shows areas that have SLF reported but have not yet been investigated. Dashed and spotted areas are where SLF is reported, but was not detected.

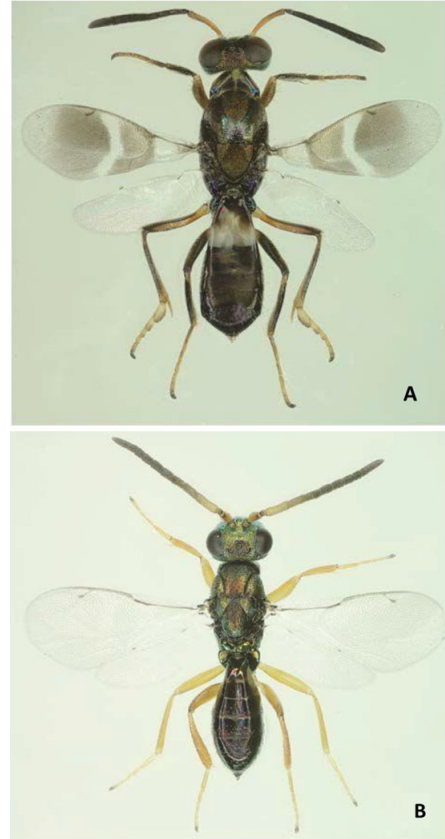
### Field surveys for SLF natural enemies

Field surveys for SLF natural enemies were initiated in October, 2015 in Beijing, Tianjin, and Liaoning China. In this first year, 115 egg masses from nine sites were collected and reared. In 2016, 286 egg masses and 529 nymphs were collected and reared from nine sites in Beijing, Tianjin, Hebei, Liaoning, Shanxi, Henan, Shandong, and Guizhou. In 2017 and 2018, surveys were made at additional sites to encompass 20 provinces. Again, egg masses and nymphs were collected. To date, an egg parasitoid, *Anastatus orientalis* (Figure 2), and a nymphal parasitoid, *Dryinus* sp. (Figure 3), have been identified as warranting additional investigation. The egg parasitoid, *A. orientalis*, is widely distributed across nearly all surveyed sites in China. It was first discovered in northern China in 2011 and has been established in Korea as a biological agent. Parasitism rates by *A. orientalis* ranged from 0-92.3% (discovered egg masses) and 0-26% (total eggs). The nymphal parasitoid, *Dryinus* sp., was found attacking second and third instar SLF nymphs. In Beijing and Shandong, apparent parasitism of second and third instar nymphs by the dryinid parasitoid was approximately 30%, although actual parasitism is likely higher.

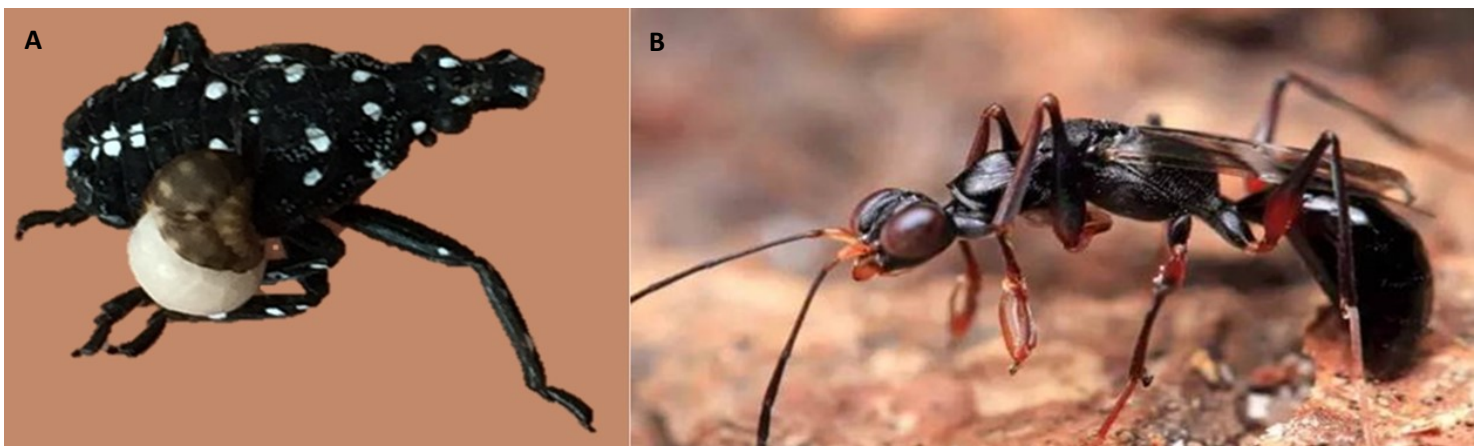
### Ongoing research

Biological control work with *A. orientalis* is ongoing and a collection of *Dryinus* from June, 2018 is being evaluated. Information on how to optimally rear both the spotted lanternfly and its natural enemies, as well as host specificity testing, are essential before natural enemies can be considered for release.

To this end, we are conducting research to evaluate the life cycle, sex ratio, longevity, fecundity, and foraging behaviors of *A. orientalis*. We are also developing optimal rearing conditions and testing its host range. With *Dryinus* sp., we have our first collection from this past summer in quarantine and this spring plan on exposing SLF nymphs to any wasps that emerge.



**Figure 2.** A) Female and B) male egg parasitoid *Anastatus orientalis* recovered from spotted lanternfly egg masses collected in China. Photos by Xiao-yi Wang.



**Figure 3.** Nymphal parasitoid, *Dryinus* sp., which attacks spotted lanternfly nymphs. A) *Dryinus* developing in a thalacium under the wing bud of an SLF nymph and B) an adult *Dryinus*.

## Optimizing the geographical locations for release of emerald ash borer parasitoids

Juli R. Gould<sup>1</sup>, Melissa Warden<sup>1</sup>, and Ben Slager<sup>2</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>EAB Biocontrol Rearing Facility, USDA APHIS, Brighton, MI

### Introduction

The emerald ash borer, EAB, *Agrilus planipennis*, was introduced into the United States sometime in the 1990s near Detroit, Michigan. As of April, 2019 populations have spread to 35 states as well as Washington, DC. After initial eradication attempts were not successful, scientists studied EAB in its native range, discovered several promising parasitic wasps, and developed a biological control program. Parasitoid releases began in Michigan and its surrounding states in 2007 with the release of two larval parasitoids, *Tetrastichus planipennis* and *Spathius agrili*, and one egg parasitoid, *Oobius agrili*.

*Tetrastichus planipennis* and *O. agrili* established in these northern states, and although *S. agrili* overwintered successfully, populations did not persist. Because a parasitoid with a long ovipositor like *S. agrili* is necessary to attack EAB in large trees, *Spathius galinae* was introduced from Russia beginning in 2015 in northern areas of the United States. Populations of *S. galinae* seem to be established and are spreading in several northern states.

Studies were initiated to discover why the various parasitoids did or did not establish in the north, and to determine if they could establish in the south, with a particular emphasis on the role played by the yearly phenology (life-cycle) of EAB and the three larval parasitoids. It was concluded that in both the northern and southern U.S., *S. agrili* emerges from diapause (suspended development) several months after EAB (i.e. mid-summer), while both

*T. planipennis* and *S. galinae* emerge as temperatures begin to warm in the spring. In southern areas like Tianjin, China where

*S. agrili* was collected, 100% of the EAB overwinter as J-larvae in overwintering chambers relatively deep in the wood, beyond the reach of the parasitoid's ovipositors. It makes sense that in such a climate *S. agrili* would wait for EAB to emerge as adults, lay eggs, and develop to the mature larval stage preferred by *S. agrili*. In northern China and the Russian Far East, where *T. planipennis* and *S. galinae* were collected, many EAB do not complete development in one year, and some proportion overwinter as larvae under the bark rather than J-larvae in overwintering chambers. These larvae are available to the parasitoids for oviposition in the spring.

EAB parasitoids are expensive to produce, and the EAB bio-control program strives to release parasitoid species only where there is a strong chance that they will establish. Therefore, *T. planipennis* and *S. galinae* are released at sites where EAB has a two-year life cycle (generally in the north) and *S. agrili* is released where EAB has a one-year life cycle (generally in the south). Because the proportions of EAB overwintering as larvae versus J-larvae from north to south was unknown, the 40<sup>th</sup> parallel was chosen as a logical dividing line. Above the 40<sup>th</sup> parallel we released *T. planipennis* and *S. galinae* and south of the 40<sup>th</sup> parallel we released *S. agrili*. This study was designed to sample overwintering populations of EAB throughout its range in the United States and to produce a more detailed model of the availability of mature EAB larvae to parasitoid adults emerging in the spring.

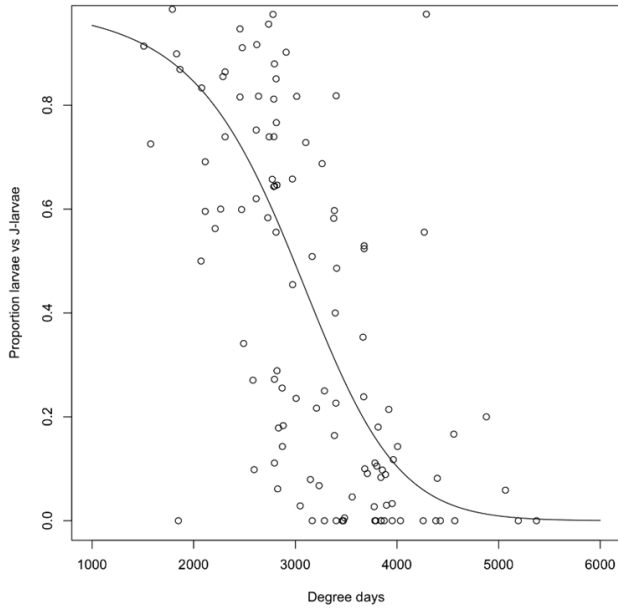
### Collection of Data on EAB Overwintering Stages

Data were collected at 69 sites in 21 states ranging from Minnesota south to Louisiana and from Colorado west to New Hampshire. At each site, cooperators were asked to fell four trees with signs of EAB: two lightly infested and two heavily infested. Many cooperators sampled more trees than requested. For each tree the cooperators recorded the number of individual EAB that were 1<sup>st</sup> to 4<sup>th</sup> instar, J-larvae, and pupae. Samples were collected in the winter of 2016/2017 and the winter of 2017/2018. All samples were collected when EAB was dormant, between October and March. We hypothesized that the most important factor determining the overwintering stage would be the temperatures experienced by EAB larvae the summer before sampling. EAB adults emerge over time and eggs are therefore laid throughout the summer. The more heat units that are accumulated in a given area, the greater the proportion of EAB that will complete development and become J-larvae. We therefore correlated the proportion of overwintering EAB that were present as larvae with the number of growing degree days at base 50°F (GDD50°F) experienced at that location between January 1<sup>st</sup> and September 30<sup>th</sup> the previous summer.

### Results

The proportion of EAB that overwintered as larvae rather than J-larvae did indeed decrease significantly as the number of growing degree days increased (Figure 1).





**Figure 1.** Logistic regression correlating the proportion of EAB that overwintered as larvae rather than J-larvae with Growing Degree Days (Base 50°F).

We used the resulting logistic regression to model the predicted percentage of EAB overwintering as larvae throughout the United States based on accumulated degree days (Figure 2). In the eastern half of the country, 25-100% of the EAB overwinter as larvae generally to the north of the 40<sup>th</sup> parallel. In areas where at least 25% of EAB overwinter as larvae, establishment of *T. planipennisi* has been excellent (red stars on Figure 2). Interestingly, *T. planipennisi* was recovered in several locations in the middle of the country (southern Indiana, Maryland, Virginia, Kentucky, Tennessee, and Missouri) where only 10-25% of the EAB overwintered as J-larvae.

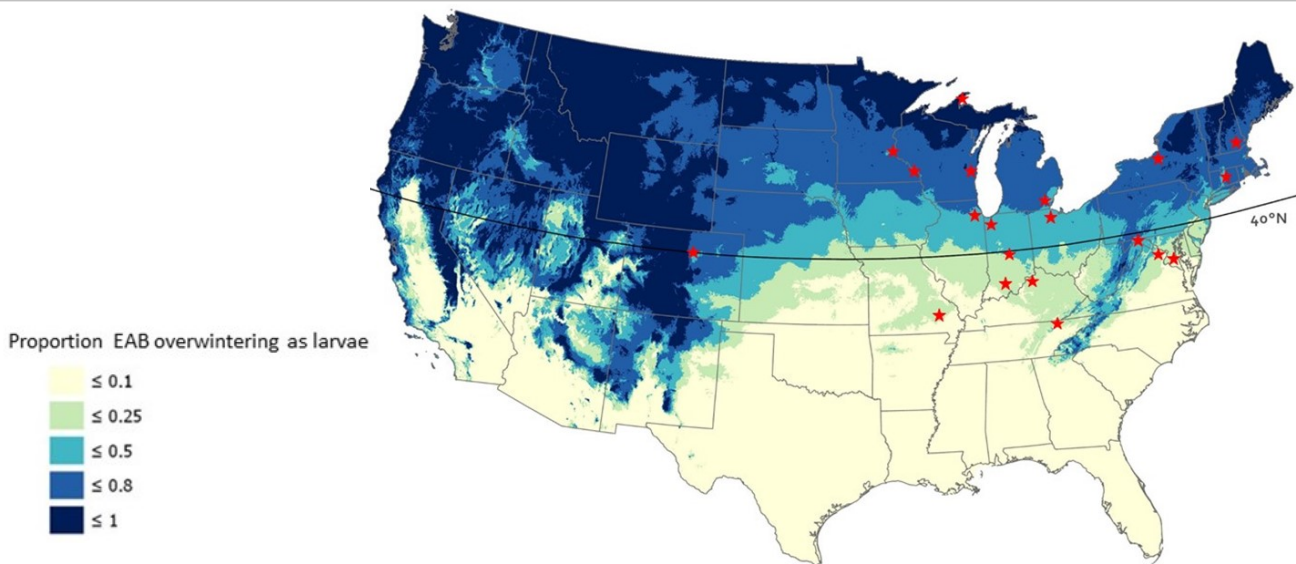
That proportion seems to be sufficient for establishment of this parasitoid species. Recovery of the parasitoids does not predict impact on EAB populations, however. With the exception of Maryland and northern Kentucky, recoveries of *T. planipennisi* in the 10-25% zone consist of only a few individuals recovered in a single year. We are continuing to study the dynamics of established parasitoids in the south to determine if established parasitoids can build to sufficient numbers to impact EAB population density and impact ash conservation over time.

As predicted, we have not recovered *T. planipennisi* at release sites where over 90% of the EAB overwintered as J-larvae. *Spathius galinae* has only been released since 2015, and releases have only occurred where 50-80% of the EAB overwinter as larvae (north of the 40<sup>th</sup> parallel). Recoveries of *S. galinae* are even more recent, but to date they have established in Illinois, New York, Massachusetts, and Connecticut.

Based on the logistic regression, one can expect 10% or more of the EAB to overwinter as larvae if fewer than 3,529 GDD50° F have accumulated between January 1<sup>st</sup> and September 30<sup>th</sup>. The degree day cutoff for 25% larvae is 2,987 GDD50° F.

**Conclusion**

To save resources we recommend that the EAB Biocontrol Rearing Facility give priority to shipping *T. planipennisi* and *S. galinae* to locations accumulating  $\leq 2,987$  GDD20°F each summer. In more southern locations priority should be given depending on whether *T. planipennisi* is persisting and impacting EAB populations.



**Figure 2.** Predicted proportion of EAB overwintering as larvae throughout the United States. Red stars indicate locations where *T. planipennisi* has been recovered.



## Assessing the host range of a potential North American biological control agent of Asian longhorned beetle

Theresa C. Murphy<sup>1</sup>, Juli R. Gould<sup>1</sup>, Xingeng Wang<sup>2</sup>, and Ellen M. Aparicio<sup>2</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>Beneficial Insects Introduction Research Unit, USDA ARS, Newark, DE

### Introduction

Asian longhorned beetle, ALB, *Anoplophora glabripennis*, is a serious invasive pest in the United States. Although currently under an extensive eradication program, it has been found in multiple states since the 1990s and remains a threat for re-establishment. Given the critical need to manage this pest, it is important to look into sustainable management practices such as biological control, which can be useful in forested areas where chemical or manual control can be prohibitively expensive and/or environmentally undesirable.

While work is ongoing to find specialized natural enemies in ALB's native range in China (classical biocontrol), research has shown that indigenous natural enemies in the introduced range of the pest can also be useful in managing invasive species (new association biocontrol). *Ontsira mellipes* is a parasitoid native to North America that has readily attacked ALB in experiments conducted in the quarantine facility at the USDA ARS Beneficial Insects Introduction Research Unit (BIIRU) and holds promise as a potential biocontrol agent (Figure 1) [1]. Unfortunately, despite conducting research on this species, its native host range remains unknown.

We investigated the host preferences of this parasitoid against native cerambycid beetles and also against citrus longhorned beetle, CLB, *Anoplophora chinensis*. While CLB is not currently found in North America, it has been intercepted at U.S. ports of entry and is considered an invasive species threat [2]. The main objectives of this study are to identify what species *O. mellipes* attacks and to determine whether *O. mellipes* prefers ALB over these species.



**Figure 1.** An *A. glabripennis* (ALB) larva being fed upon by larvae of *O. mellipes*.

### Methods

Testing was done at the USDA ARS BIIRU. BIIRU maintains ALB, CLB, and the parasitoid (*O. mellipes*) in colonies under controlled conditions (23°C, 16L:8D).

Native beetles were live-trapped using multifunnel and intercept traps, both baited with multi-cerambycid and ethanol lures, and light traps. Trapping was conducted from May to August, 2018 in northern hardwood forests in Massachusetts near Otis Laboratory. For details regarding collection, mating, egg harvesting, and larval rearing methods see Gould et al. 2018 [3]. Adult beetles were fed and allowed to mate and oviposit; eggs were sent to BIIRU for larval rearing on artificial diet and parasitoid testing. Six species (*Elaphidion mucronatum*, *Monochamus carolinensis*, *Monochamus notatus*, *Neoclytus scutellaris*, *Xylotrechus colonus*, and *Xylotrechus sagittatus*) produced enough eggs (>150) and enough larvae (>30) for testing. *Elaphidion mucronatum* and *X. colonus* attack several species of eastern hardwoods. *Monochamus carolinensis* and *M. notatus* are common wood-boring beetles of pine and are economically important pests as they can be vectors of the pinewood nematode, *Bursaphelenchus xylophilus*. *Neoclytus scutellaris* prefers to attack oak but can also feed on pine.

Choice tests were conducted on similar size larvae in square ventilated containers in growth chambers (22°C, 14L:10D). Bark flaps were created to insert larvae into sticks (for details on this method see Golec et al., 2018 [4]). ALB, CLB, *E. mucronatum*, and *X. colonus* were inserted into Red maple (*Acer rubrum*) while *X. sagittatus*, *M. carolinensis*, *M. notatus*, and *N. scutellaris* were inserted into Virginia pine (*Pinus virginiana*). Test female *O. mellipes* were reared from ALB. For each choice test, two sticks were provided: one with an ALB and the other with a test larva. Two mated female *O. mellipes* that had not previously been exposed to a host were released into the containers for one week. Honey and water were provided for the parasitoids. Three weeks after each exposure, sticks were dissected to identify the presence of developing parasitoids or signs of parasitism on the larvae. *Neoclytus scutellaris* is excluded from the choice test results because there were only four replicates due to difficulties in rearing larvae.

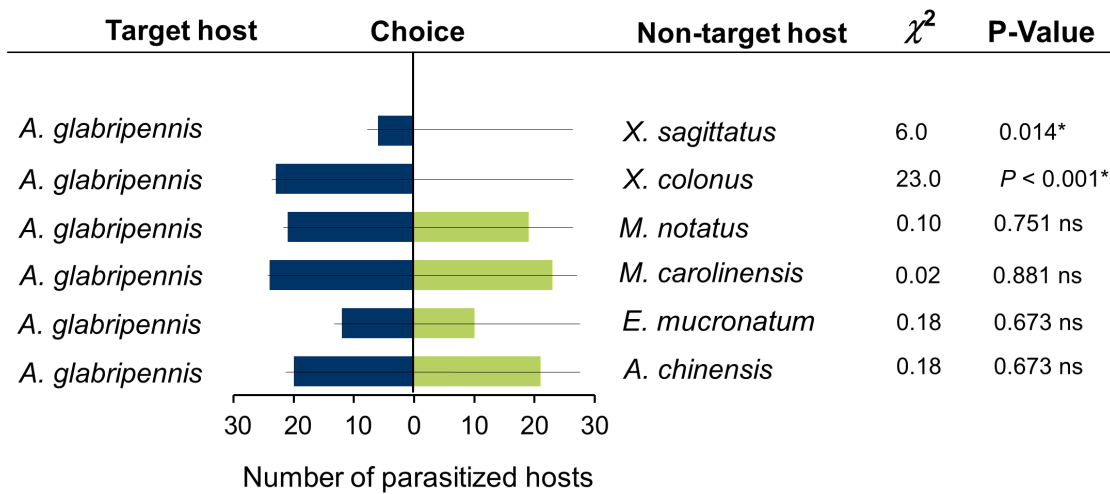
**Results**

*Ontsira millepes* attacked *M. carolinensis*, *M. notatus*, *E. mucronatum*, ALB and *A. chinensis*, but did not attack *X. colonus* and *X. sagittatus* (Figure 2). The parasitoid did not show a preference between native cerambycids and ALB (Figure 2). *Ontsira millepes* was observed searching and ovipositing on infested sticks of three native species (*E. mucronatum*, *M. carolinensis*, and *M. notatus*) and two Asiatic species (ALB and CLB). These behaviors were not observed on the two unparasitized native hosts (*X. colonus* and *X. sagittatus*).

**Discussion**

*Ontsira millepes* appears to be a true generalist, adapted to a broad range of species. Wood-boring parasitoids often use chemical or physical cues produced by its host during feeding and/or defecating. Given that *O. mellipes* attacked five different species in two different trees, it is likely attracted to cues released by a range of related species. We speculate that *O. mellipes* was either not attracted to or was unable to detect the external or physical cues produced by the species that were not attacked.

Though the introduction of specialist parasitoids is ideal, research has not yet identified any natural enemies that are proven specialists for ALB. *Ontsira millepes* is the only viable option so far and may still be an important parasitoid for the biocontrol of ALB. It has demonstrated the ability to adapt to new host species like ALB and CLB. Furthermore there is evidence that the risk of parasitism can increase for one host when it shares other hosts [5]. This may be applicable to *O. millepes* since it is just as likely to attack ALB as any of the native species tested. Our testing also indicates that *O. millepes* can be easily mass reared on a native host such as *M. carolinensis* outside of quarantine facilities. Further studies to assess whether or not *O. millepes* could be useful in augmentative or conservation biocontrol to regulate ALB are warranted. Next steps would include studies to evaluate the efficacy of *O. mellipes* in suppressing ALB populations under field conditions (cage and/or open release) within the quarantined area of this pest in the United States.



**Figure 2.** A dual-choice test showing the number of the target host (*A. glabripennis*) and the alternative host species parasitized by *O. mellipes*. Tested wasps were reared from *A. glabripenni*. Asterisks indicate significant difference (Chi-square test; ns = not significant).

**References**

- 1) Golec JR, Duan JJ, Aparicio E, Hough-Goldstein J. Life history, reproductive biology, and larval development of *Ontsira mellipes* (Hymenoptera: Braconidae), a newly associated parasitoid of the invasive Asian longhorned beetle (Coleoptera: Cerambycidae). *Journal of economic entomology*. 2016 Jun 21;109(4):1545-54.
- 2) Haack RA. Exotic bark-and wood-boring Coleoptera in the United States: recent establishments and interceptions. *Canadian Journal of Forest Research*. 2006 Feb 1;36(2):269-88.
- 3) Gould JR, Aflague B, Murphy TC, McCartin L, Elkinton JS, Rim K, Duan JJ. Collecting Nontarget Wood-Boring Insects for Host-Specificity Testing of Natural Enemies of Cerambycids: A Case Study of *Dastarcus helophoroides* (Coleoptera: Bothriideridae), a Parasitoid of the Asian Longhorned Beetle (Coleoptera: Cerambycidae). *Environmental entomology*. 2018 Sep 5;47(6):1440-50.
- 4) Golec JR, Li F, Cao L, Wang X, Duan JJ. Mortality factors of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) infesting *Salix* and *Populus* in central, northwest, and northeast China. *Biological Control*. 2018 Nov 1;126:198-208.
- 5) Holt RA, Lawton JH. The ecological consequences of shared natural enemies. *Annual review of Ecology and Systematics*. 1994 Nov;25(1):495-520.

## Efficacy of surface treatments in preventing hatch from spotted lanternfly egg masses

Phil Lewis<sup>1</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

### Introduction

Spotted lanternfly, SLF, *Lycorma delicatula*, a sap feeding insect native to Taiwan and China, was first detected in the United States in 2014. This pest has the potential to impact a number of Pennsylvania's commodities, including grape, apple, and stone fruit (\$180 million annually) as well as pine and hardwood lumber sales (\$12 billion annually). The pest has been detected in multiple counties in southeastern Pennsylvania, as well as areas in New Jersey, Delaware, and Virginia that border the Pennsylvania infestation. Control efforts are ongoing and include outreach and education activities, host tree removal, and chemical treatments of tree of heaven, *Ailanthus altissima*.

SLF adults can lay their eggs on almost any flat surface; this includes not only the bark of their preferred host, but also lawn furniture, rocks, fence posts, firewood, and other similar places. A regulatory treatment that could prevent SLF hatch would be an important tool for population suppression as well as a key component in facilitating the safe movement of commodities, nursery stock, household articles, transport vehicles, etc. outside of the quarantine zone.

### Methods

USDA's Otis Laboratory tested a number of pyrethroid insecticides and two dormant oil sprays to determine if these surface treatments would be effective in reducing or eliminating SLF egg hatch. Individual egg masses (Figure 1) were chiseled from tree trunks in early April, 2018, randomly placed into groups of 20, and exposed to the various treatments (see Table 1 for additional information).



**Figure 1.** Spotted lanternfly egg masses used in this study.

Egg masses were sprayed to run-off using a 24-ounce hand pump sprayer, allowed to dry, placed into individual petri dishes, and transported back to an incubator in our quarantine facility.

Egg masses were maintained in the incubator at 25°C, 65% humidity, 16:8 light cycle, and checked regularly until first hatch was observed near the end of April.

### Results

We observed the hatch of viable SLF nymphs from all of the surface treatments except for the Golden Pest Spray Oil treatment where none emerged successfully (Table 1). The other treatments had an average hatch that ranged from 5.9 to 21.8 nymphs per egg mass. The control treatment had a total of 47 nymphs hatch from the 20 egg masses for an average of 3.7 nymphs per egg mass.

There were significant differences between the treatment groups; ANOVA results of the log transformed data were significant ( $F_{1,139} = 14.14, p < .001$ ) and pairwise comparisons using Tukey HSD are shown in Table 1.



**Table 1.** Treatment information and emergence numbers of SLF nymphs following exposure to various surface treatments. Twenty egg masses collected in early April, 2018 were used for each treatment. Values in the last column followed by the same letter are not statistically different (ANOVA and Tukey HSD where  $F_{1,139} = 14.14, p < .001$ ).

Egg mass treatments	Rate applied	SLF nymphs surviving hatch	Masses with zero emergence	Avg SLF nymphs hatching /mass
Talstar P (bifenthrin)	0.06%	158	10	7.9 C
Tempo SC Ultra (β-cyfluthrin)	0.005%	435	2	21.8 A
Demand SC (lambda-cyhalothrin)	0.5 oz/gal	191	8	9.6 BC
Onslaught (esfenvalerate)	0.05%	385	2	19.3 AB
Golden Pest Spray Oil (soy)	50%	0	19	0.0 D
Bonide Dormant Oil (petrol)	3%	117	5	5.9 CD
Control (water)	N/A	47	8	3.7 CD

**Discussion**

The pyrethroid products tested in this study suppressed emergence to varying degrees, but the Golden Pest Spray Oil prevented emergence of viable SLF nymphs. This work should be repeated as the control group had poor egg hatch.

There is limited information on SLF control in the scientific literature. Researchers in South Korea tested various products from six major insecticide classes, insect growth regulators and machine oil and found that chlorpyrifos resulted in 94% or greater mortality of SLF hatching from treated egg masses [1]. Far less control was observed when field-collected egg masses were treated in May, right before hatch. None of the other insecticides tested showed much activity, with corrected mortality ranging from 0 to 37%, although only two to three egg masses were used to assess each treatment.

Further testing to confirm and improve on these results is planned. Our laboratory will modify some application rates and test products from other insecticide classes. We will also investigate if the dark gray material that female SLF secrete over their eggs (Figure 2) provides protection. Fall and spring applications will be made to determine if successful egg hatch is influenced by treatment timing.



**Figure 2.** Freshly laid SLF egg masses

**Reference**

- 1) Shin Y, Moon S, Yoon C, Ahn K, Kim G. Insecticidal activity of 26 insecticides against eggs and nymphs of *Lycorma delicatula* (Hemiptera: Fulgoridae). Dept. of Plant Medicine, College of Agriculture, Life, and Environment Sciences, 2010. Chungbuk National University, Cheongju, Korea (Translated 1/26/15).

## Using sentinel trap trees to detect spotted lanternfly

Phil Lewis<sup>1</sup> and Amanda Davila-Flores<sup>1</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

### Introduction

Spotted lanternfly, SLF, *Lycorma delicatula*, a sap feeding insect native to Taiwan and China, was first detected in the U.S. in 2014. Field populations continue to expand beyond the 13 county quarantine in southeastern Pennsylvania. Severe impacts by this insect have been noted.

There is a need for a sentinel trap trees that can be easily deployed in states adjoining the SLF infestation so that any expansion of this pest insect can be quickly identified. This insect has a high likelihood of transport due to its movement propensity and because egg masses are laid on almost any vertical surface. The preferred SLF host is tree-of-heaven, *Ailanthus altissima*; this tree is found in disturbed sites and is very common along roadways and median strips in south-eastern Pennsylvania. Key transportation routes, railways, and DOT facilities are ideal locations for setting up sentinel trap trees. Additional areas of concern such as vineyards and farmlands warrant monitoring with this method. SLF is a very active and mobile insect and is highly attracted to *Ailanthus*, especially at the adult stage. Therefore the attract and kill method is a good means of detecting low population levels of SLF in an area compared to occasional visual inspection.

### Method

Sentinel trees (between 4 and 10" in diameter) were established by selecting areas of interest/ concern that had *Ailanthus* trees that were both readily and safely accessible from roadways. Trees were treated with a bark spray application using the maximum label rate of dinotefuran (Figure 1).



Figure 1. Spray application.

A May/June treatment schedule was used, based on prior research conducted at Otis Laboratory that showed the treatment remained effective against this insect through the fall. Following the pesticide application, a tree watering ring was placed around the base of the sentinel tree to catch SLF life stages that were killed by the insecticide (Figure 2). The watering ring is self-draining. The sentinel trees were monitored every two to three weeks or more often as personnel were available. Data on trap catch was reported via a modified version of the Collector for ArcGIS application (ESRI, CA, USA).



Figure 2. Sprayed tree with collection ring. Spray will dry within 1/2 hour.

### Results

Participants in 2018 included state and federal cooperators in Pennsylvania, New Jersey, Virginia, and Maryland. Traps were checked every two weeks, on average. Pennsylvania and Maryland deployed 28 and four traps, respectively. In Pennsylvania, traps were established on the western edge of the core infested area. However, no detections were made in the Maryland or Pennsylvania traps during the field season. Virginia deployed five traps, and 25 detections were made—mostly early and late instar stages and one adult. It should be noted that these traps were deployed within the area of their initial infestation site where detections would be expected.

New Jersey deployed 15 traps (Figure 3), and four of the traps yielded 10 adult SLF detections. During a search at Trap #2 a single egg mass on the tree trunk was identified (Table 1).



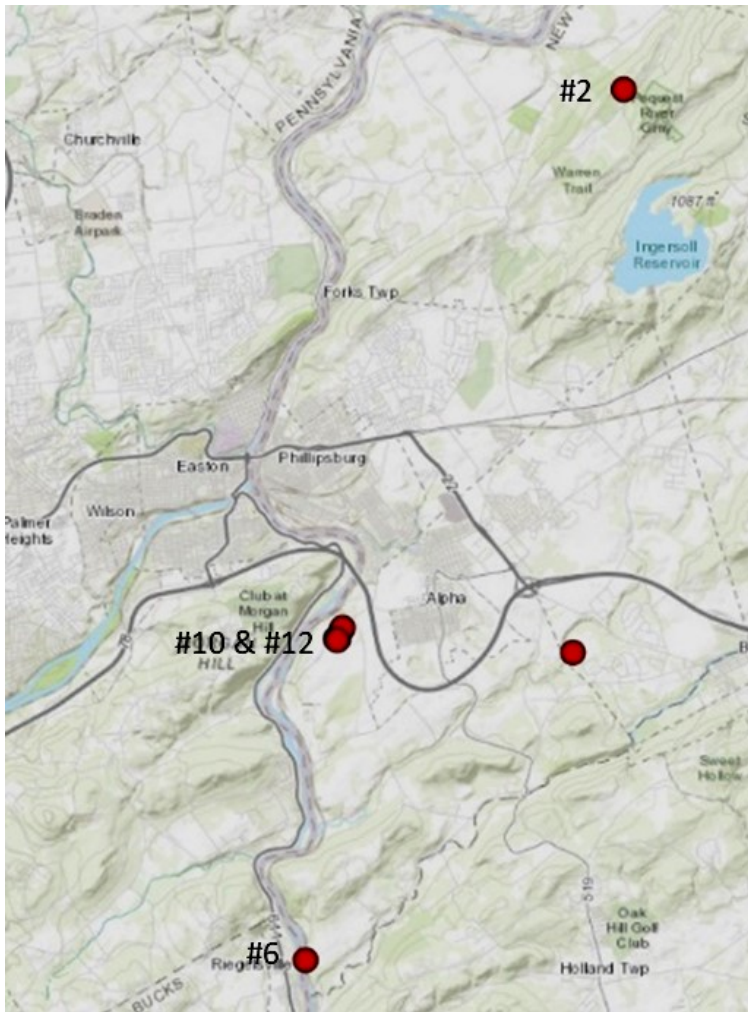
**Discussion**

Sentinel traps can be a useful tool when surveying for the presence of SLF life stages where they are not known to be present. The absence of detections (e.g., Pennsylvania and Maryland) provide support that the infestation is yet to be established in those areas.

New Jersey PPQ generally chose their trap locations based on reports from the public, locations just outside a known infested area, or in areas of concern (e.g., a vineyard, farmlands). Some of the traps were placed as a result of SLF sightings and led to further detections and expansion of the known SLF infestations. One trap was placed following a property owner’s report of an adult SLF but led to no detections, helping to confirm an isolated incident.

New Jersey used the sentinel traps to make decisions on whether to expand known areas of infestation or to note them as regulatory incidents. In the case of the trap placed on the forested edge of a commercial vineyard (Trap #6), it resulted in a positive detection and additional SLF infested trees were discovered. Control operations, which included herbicide and insecticide sprays were then initiated.

Results from our efforts in 2018 demonstrate that sentinel trap trees are most useful when deployed on *Ailanthus* outside of known infested areas. These traps can be effective as a means of investigating isolated incidents or for detecting SLF range expansion, as compared to an intense survey activity like sticky bands that must be replaced regularly. This can greatly reduce the amount of time and personnel needed by an agency as they manage an SLF infestation.



**Figure 3.** Map of New Jersey traps with positive detections noted by number.

**Table 1.** New Jersey traps with positive detections.

Trap ID	Egg mass count	Early instar count	4 <sup>th</sup> instar count	Adult count	Total
2	1	0	0	3	4
6	0	0	0	2	2
10	0	0	0	4	4
12	0	0	0	1	1

## Application methods to control spotted lanternfly

Phil Lewis<sup>1</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

### Introduction

Spotted lanternfly, SLF, *Lycorma delicatula*, a sap feeding insect native to Taiwan and China, was first detected in the U.S. in 2014. Field populations continue to expand beyond the 13 county area currently under quarantine in southeastern Pennsylvania. Severe impacts by this insect have been noted.

The current control measure being employed by state and federal cooperators against SLF is a bark spray application of dinotefuran on the preferred host, tree-of-heaven, *Ailanthus altissima*. The effectiveness of this control approach is readily apparent, especially later in the year when dead SLF adults can be found piled at the base of treated trees (Figure 1).



**Figure 1.** Dead and dying SLF adults at the base of a treated tree.

### Methods

Various insecticide and application methods were compared during the 2017 and 2018 field seasons to determine optimal treatments and to verify efficacy. Dead SLF adults were counted on tarps positioned under the dripline of treated trees over a six week period from late August to early October. Tarps were checked and cleaned every two to three days during testing (Figure 2).



**Figure 2.** Tarp layout used to monitor SLF mortality following treatments.

Trunk injection treatments of dinotefuran, imidacloprid (Dinocide & Imicide 10% formulations; JJ Mauget) and emamectin benzoate (Tree-age; Arborjet) were compared to the standard SLF program treatment of a bark spray application of dinotefuran (Safari or Transtect; Valent, Rainbow Scientific) using the maximum label rate. Injection treatments were applied at 2 mL of formulated product per diameter inch of tree. In 2017 there were four trees treated with bark spray and two trees for each of the trunk injectable products. In 2018 the number of trees used for each injectable product was increased to five and the emamectin treatment was dropped. All study trees were deep root fertilized out to the dripline with ArborGreen Pro 30-10-7 in June of 2018 by Davey Tree.



**Results**

The emamectin product was only tested in 2017 as mortality on most sample dates was not very different from the control group. However, adult SLF are susceptible to both dinotefuran and imidacloprid applied as either an injectable formulation or as a bark spray (Figure 3). In 2017 there were about 28,000 dead SLF adults collected from 12 tarps during the study. Populations increased almost 10-fold the following year as there were over 252,000 dead SLF adults tallied from under 17 study trees (same time period and research site).

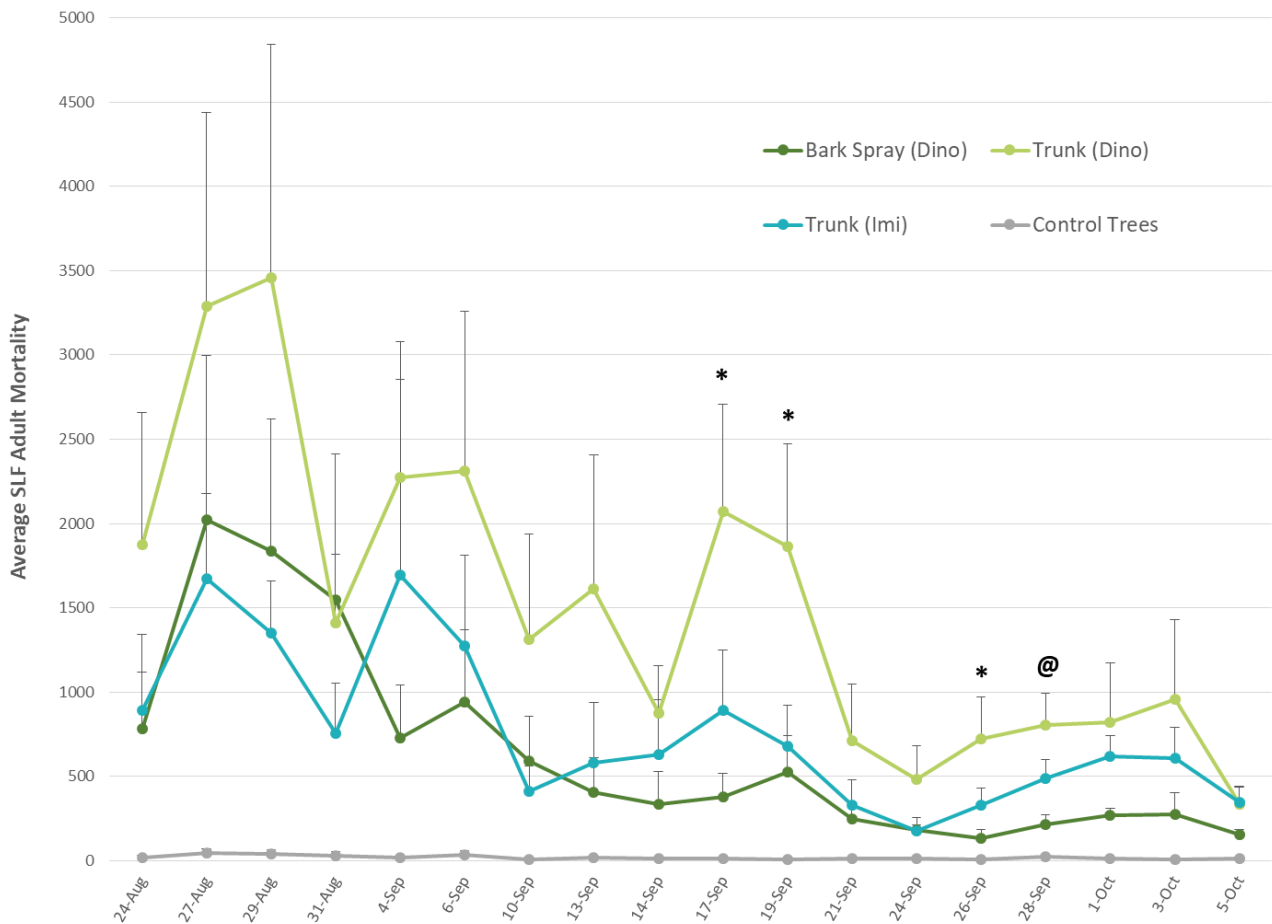


**Figure 3.** Dead SLF adults on a tarp.

Mortality data were highly variable over time (Figure 4) and also varied greatly by individual tree, with some trees attracting and killing a disproportionate number of SLF. A single tree in 2017 accounted for about 36% of the catch and three of the most productive trees in 2018 made up 52% of the total (Figure 5). The three most productive trees in 2017 were again among the most productive in 2018.

**Discussion**

Because of the variability in tree attractiveness in 2017, all study trees were fertilized in 2018 to see if levels of nitrogen, phosphorus, and potassium (NPK) play a role in attracting SLF adults. Tree foliage was analyzed prior to fertilization and monthly between August and October. There were no significant differences in the NPK values of individual trees or over time from the four sample collections taken. Values also did not differ between the study trees and nearby, untreated *Ailanthus* trees, so nutrient levels do not appear to be a factor in how SLF adults are selecting certain trees.



**Figure 4.** Average SLF adult mortality by date over a 6 week period in 2018.



Spotted lanternfly seem to be susceptible to most insecticides and were readily killed after feeding on trees treated with either dinotefuran or imidacloprid. It is evident that these insects move around a lot, as SLF adults were coming in from untreated trees on the property and dead insects were continually landing on the tarps over the six week period. Standard errors noted in Figure 4 highlight the extreme variability in catch within treatment groups as there were only three dates where the Dinocide treatment was different from the control ( $p < 0.05$ ; asterisk) and a single instance where Dinocide was superior to the bark spray treatment ( $p < 0.01$ ; ampersand).

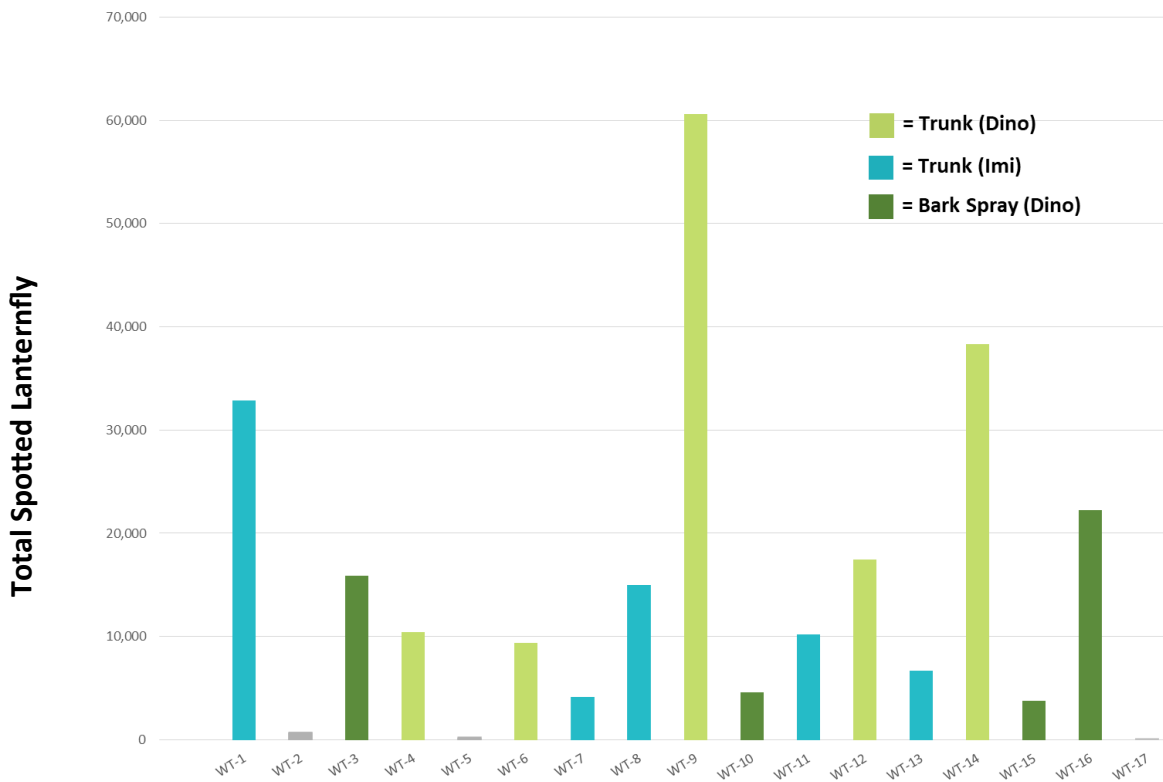
Data from 2017 had similar trends and it appears that SLF adults are more susceptible to the Dinocide injection treatment and/or are killed faster and therefore more SLF end up on the tarps. The variability in SLF adult catch among individual trees in 2018 is displayed in Figure 5 and was similar to results from 2017. Three trees caught over half the SLF adults over a six week period in the 2018 tests, so it appears that some factor in certain *Ailanthus* trees draws or retains them.

Two years of observations on the same property noted certain trees that were more heavily populated by SLF adults and this was consistent year to year. Others working with this insect have also observed that SLF prefer certain *Ailanthus* trees on a property.

Another aspect of SLF adult behavior is the reduction in SLF mortality numbers by mid-September (Figure 4). The Behavior and Chemical Ecology Laboratory has noted that SLF females become reproductively mature and begin to disperse for egg laying at about this time.

**Conclusion**

Spotted lanternfly are readily killed by two systemic insecticides applied by trunk injection or bark spray methods. The trunk injection dinotefuran treatment consistently resulted in higher mortality, but extreme variation within each set of treated trees prevented statistical separation among the treatments. Related to this, it is apparent that SLF prefer to colonize certain trees, and appear to do so year to year. Although it is unclear what drives this behavior, additional work should be pursued to elucidate tree selection behavior by this insect.



**Figure 5.** Total SLF adults collected under individual trees in 2018. Gray bars represent controls.

## Trapping male moths of *Lymantria* species in China

Baode Wang<sup>1</sup>, Zhichun Xu<sup>2</sup>, and Feiping Zhang<sup>3</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>Beijing Forestry University, Beijing, China

<sup>3</sup>Fujian Agricultural and Forestry University, Fuzhou, China

### Introduction

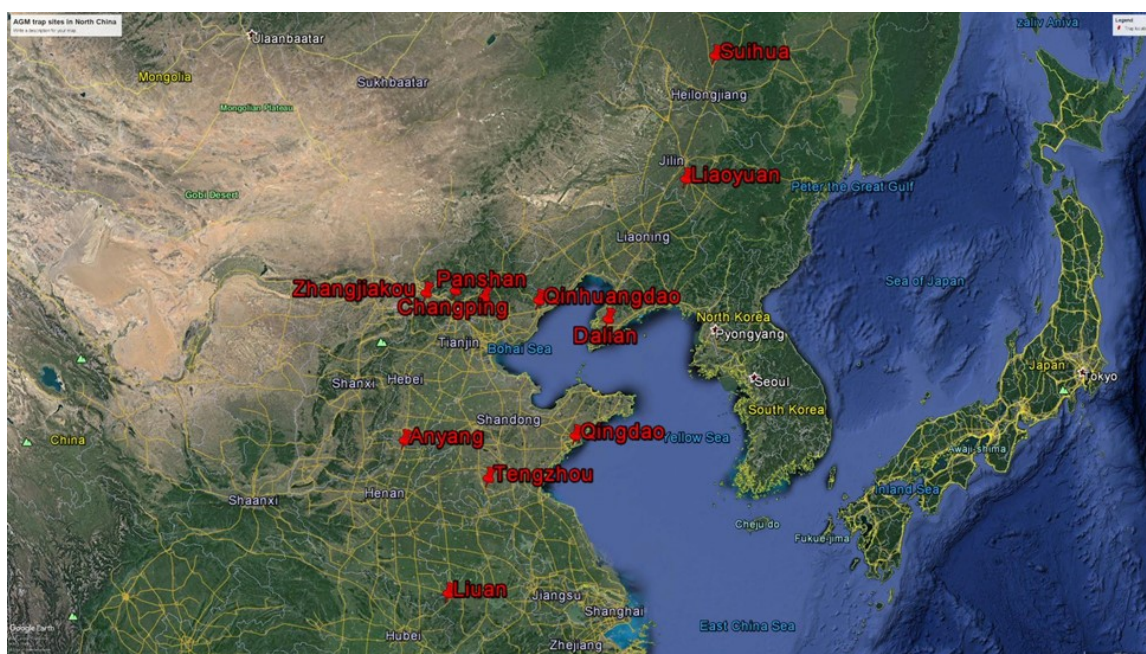
Egg masses of several species of *Lymantria* have been intercepted on vessels at ports in the United States and Canada. Male moths of *Lymantria dispar asiatic*, referred as LDA hereafter, have also been captured in traps baited with disparlure at several locations in the U.S. Unlike female moths of *L. dispar dispar* (referred to as LDD), LDA females are capable of flying, and their larvae have wider host ranges and adapt better to new environments. Egg masses of *L. mathura*, *L. monacha*, and *L. xyliina* have also been intercepted in recent years on vessels called at U.S. ports. These species, especially, the Asian gypsy moth, AGM, which includes LDA, are targets for survey and detection efforts as well as North American offshore AGM free vessel certification programs.

One of our goals is to understand AGM population levels in different areas as well as population cycles in different years so that resources can be better allocated by inspection agencies. Additionally, this information will help to improve risk mitigation measures such as the suppression of populations at the port areas in native countries where higher populations have occurred.

### Methods

In areas north of the Yangtze River in China in 2018, individual milk carton traps were baited with the following lures and placed at different locations: disparlure primarily for LDA, mathuralure for *L. mathura*, and monachalure for *L. monacha* (Figure 1). Traps were placed about a week prior to the estimated flight start of each target species. The number of male captures of each species in traps was checked every two to three days. Trapping was concluded when target insects were not captured for three consecutive checks

In areas south of the Yangtze River, milk carton traps baited with disparlure or xylinalure were placed at 13 sites in seven provinces (Fujian, Guangdong, Guangxi, Hubei, Jiangxi, Sichuan, and Zhejiang) and in Chongqing, one of the four central government controlled municipalities in China (Figure 2). Traps baited with mathuralure were only placed in Zhejiang and Fujian, while traps with monachalure were placed in Fujian only. Traps were checked once every week until no moths were captured for three consecutive checks.



**Figure 1.** Trap locations in areas north of the Yangtze River in China (in red).



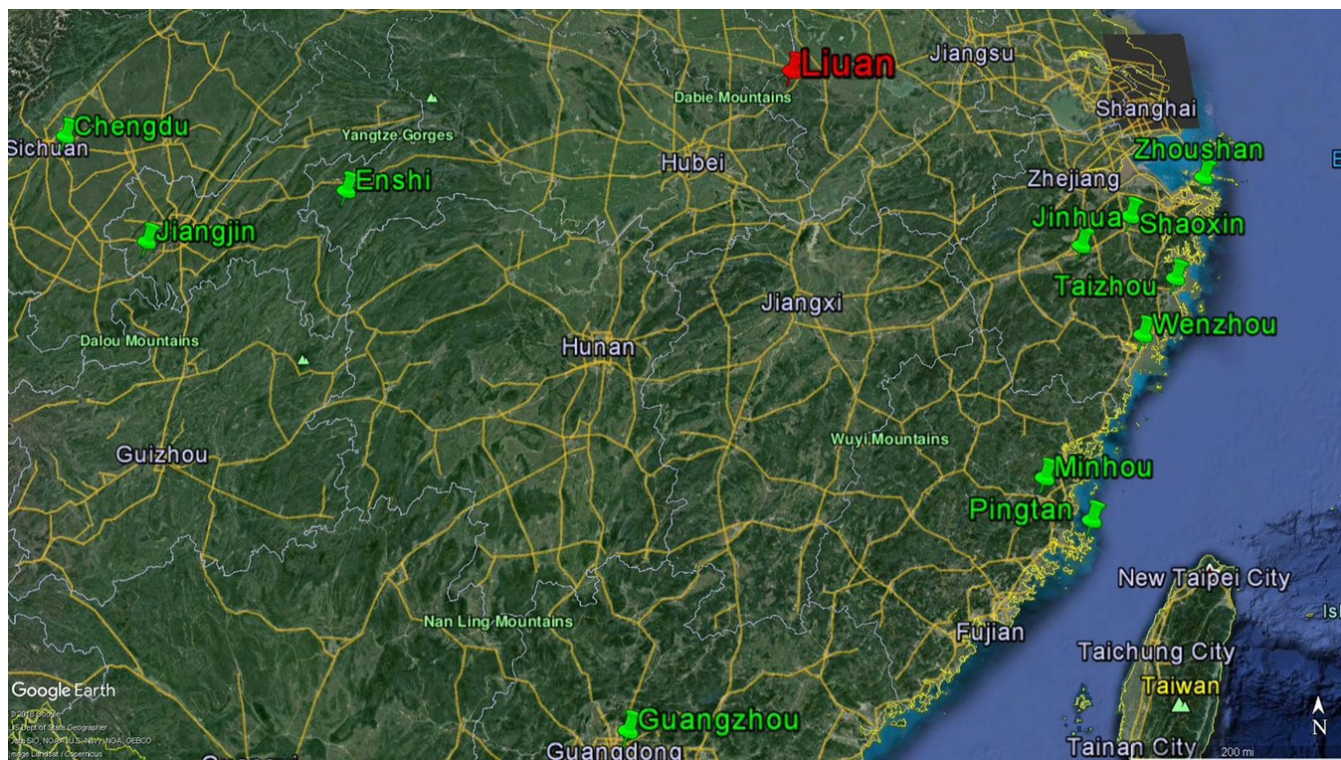


Figure 2. Trap locations in areas south of the Yangtze River in China (in green).

## Results

In areas north of the Yangtze River, traps baited with methylure and monachalure captured a few male *Lymantria* adults; however they were LDA and not *L. mathura* or *L. monacha*. The number of trap captures of LDA varied depending on the trapping site, but was generally lower than that in 2015 and 2016 for most areas (Figure 3). Trap capture in Dalian was less than previous years. Qingdao is another port city, where LDA laid egg masses on vessels at port, which were later intercepted at vessels entering the U.S. In Qingdao trap capture has been increasing in recent years. In 2018, however, trap capture in Qingdao was slightly lower than that of 2016 and 2017, but still remained high. Trap capture of LDA in the port area of Qinhuangdao remained low. Moth captures in Sihua, Liaoyuan, and Liuan in 2018 were significantly lower than that observed from 2014 to 2016, indicating declines of LDA populations in these areas in 2018.

In areas south of the Yangtze River, 6,946 *Lymantria* moths, which include LDA, *L. xyliana*, *L. dissoluta*, and *L. marginata*, were captured in traps in 2018. Among them, 6,742 were LDA caught in traps baited with disparlure at sites in Hubei, Sichuan, and Chongqing.

There was no capture of LDA at sites in Fujian, Jiangxi, Zhejiang, Guangxi, and Guangdong. Traps baited with xylinalure in Fujian and Zhejiang captured 89 *L. xyliana* male adults. For LDA male adults, three times more moths were caught in 2018 than in 2017 in Sichuan, while fewer were caught in Hubei and Chongqing. Ten traps baited with xylinalure were placed at the Pingtan Island of Fujian on May 27, 2018. However, only nine *L. xyliana* male adults were captured during the entire flight season. The other 10 traps placed at Minhou in Fujian at the same time captured 22 *L. xyliana* male moths. No *L. mathura* or *L. monacha* were found in any traps.

## Conclusions

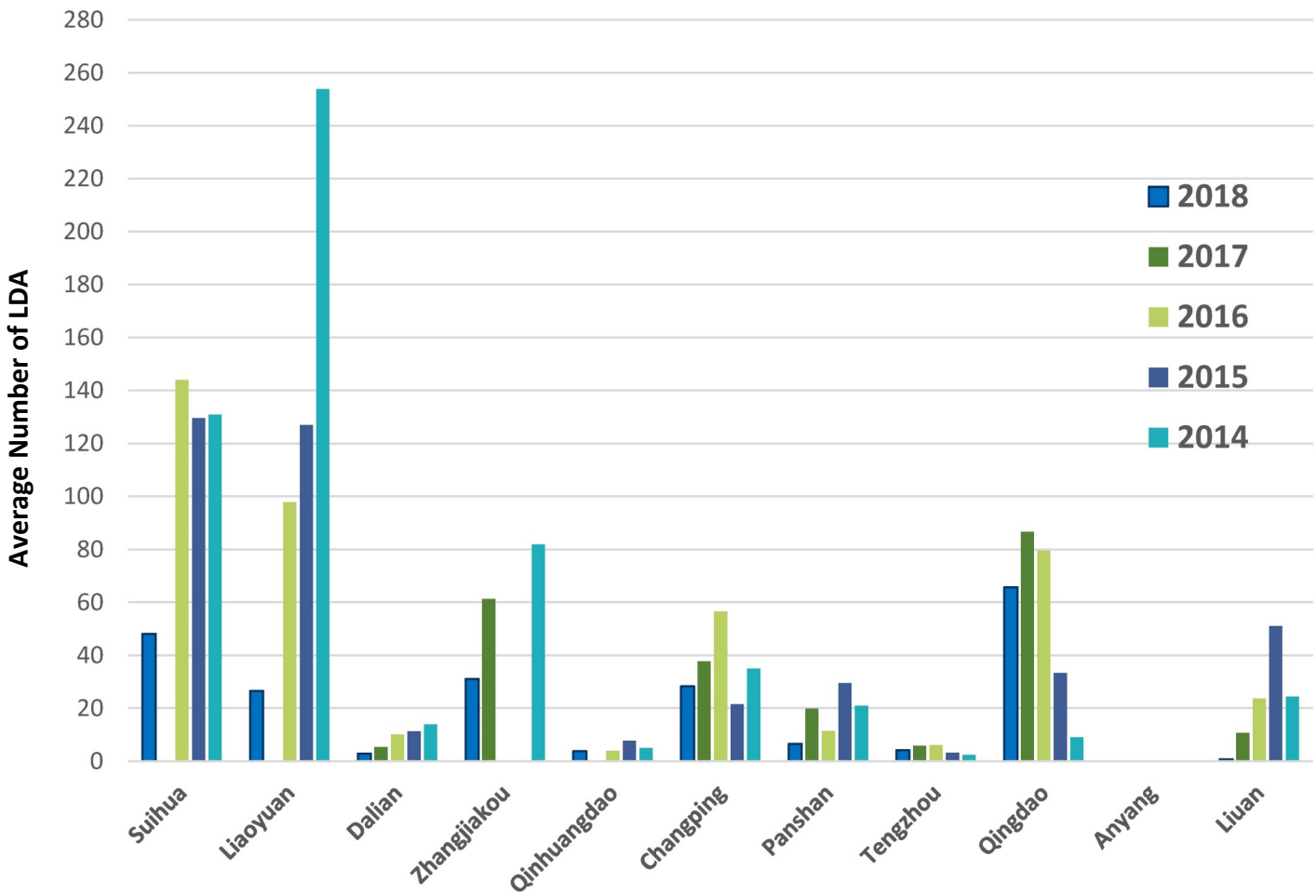
The results showed that traps baited with disparlure were able to capture not only LDA, but also *L. dissoluta* and *L. marginata*, while traps baited with xylinalure captured male adults of *L. xyliana* only. The reason for the sharp decrease in the density of *L. xyliana* in Pingtan was likely due to the effective management measures implemented in 2018, such as the application of pesticides. Further analyses are necessary to understand the increased trap capture in Sichuan.

In summary, population levels of LDA vary in different areas and in different years in China. Except for the site in Sichuan province, trap capture of LDA was lower in 2018 than in 2016 and 2017 for most areas. There were no captures of *L. mathura* and *L. monacha* at any site in China during our study. One reason for this might be the low population levels of both species at sites where traps were placed. Another reason could be that the lures and traps used for the two species were not the most effective. For example, the lure used for *L. monacha* should include three components: disparlure, monachalure, and 2me-Z7-18Hy. Additionally, instead of milk carton traps, Unitrap should be used to capture *L. monacha* [1].

Several species of *Lymantria* including *L. dispar asiatica*, and *L. xyliana* in southern China can be captured using the disparlure-baited traps. However, xylinalure is more attractive to *L. xyliana* than disparlure. Trap capture data in recent years does not seem to indicate natural population cycles of these two species; this is likely the result of the management practices in place in different areas of China.

**Reference**

- 1) Wang P, Chen GF, Zhang JS, Xue Q, Zhang JH, Chen C, Zhang QH. Pheromone-trapping the nun moth, *Lymantria monacha* (Lepidoptera: Lymantriidae) in Inner Mongolia, China. *Insect science*. 2017 Aug;24(4):631-9.



**Figure 3.** Average number of LDA male adults captured per trap at different sites.



## Woodborers attacking North American trees in China

Baode Wang<sup>1</sup>, Fangxiao Wang<sup>2</sup>, Xinsheng Cheng<sup>2</sup>, Jiabao Hong<sup>2</sup>, and Ruitong Gao<sup>3</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

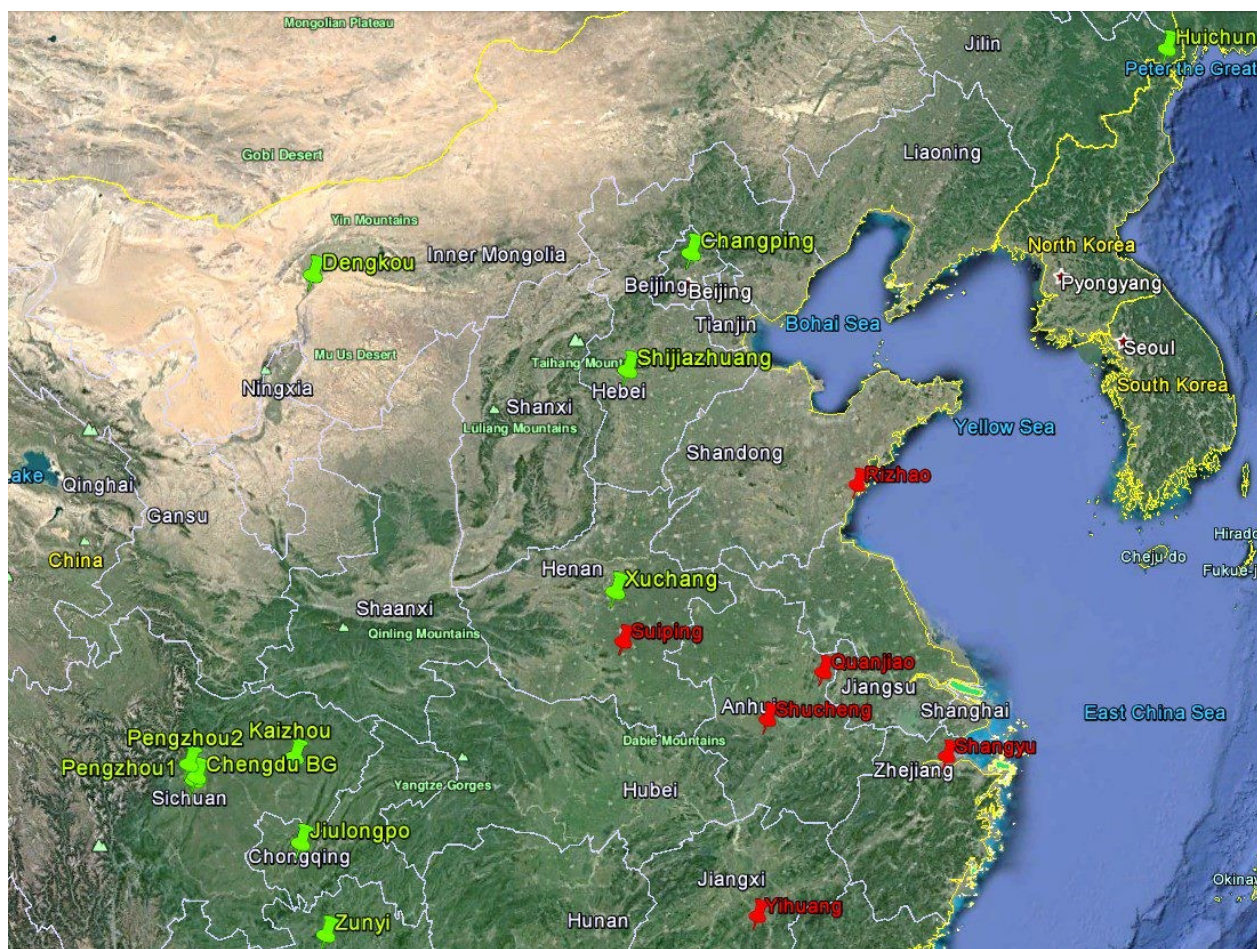
<sup>2</sup>University of Science and Technology of China, Hefei, China

<sup>3</sup>Chinese Academy of Forestry, Beijing, China

### Introduction

Quite a few insect pests that have established and caused serious damage in the United States are native to China. Two examples are emerald ash borer, EAB, *Agrilus planipennis*, and Asian longhorned beetle, ALB, *Anoplophora glabripennis*. Because some pests are not of concern in their native countries, aspects of their biology and behavior as well as management measures may not be available when they are found in new territories. In order to assess the susceptibility of North American tree species, the degree of attack and the outcome of infestations by insect pests were observed on trees planted in arboreta, as shade trees, or in commercial plantings in China.

Identifying and understanding the biology of potentially invasive insects found on North American trees in China may help to mitigate risks and improve management methods if the insect is detected in the U.S. in the future. Field surveys were conducted from 2017 through 2018 in several provinces and municipalities of China to identify insect species, especially woodborers, attacking North American trees. Street trees, shade trees, and trees from nurseries and botanical gardens were surveyed across eastern China (Figure 1).



**Figure 1.** Nursery sites where North American trees were surveyed for woodborer infestation in eastern China (red). Street, shade tree as well as trees in botanical gardens and parks were also checked for woodborer infestation in other provinces or municipal cities (green).



## Results

### Nursery trees in eastern China

Major findings based on observations of 21 tree species:

- Both ALB and Citrus longhorned beetle, CLB, *Anoplophora chinensis*, infest boxelder, *Acer negundo*, red maple, *Acer rubrum*, and Norway maple, *Acer platanoides*. More red maple trees were infested by ALB, while more boxelder and Norway maple trees were infested by CLB.
- CLB infestation were found on the following species: *Carya illinoensis*, *Cercis canadensis*, *Cotinus coggygria* 'Royal Purple', *Fraxinus americana*, *Lagerstroemia indica*, *Malus x micromalus* 'American', and *Platanus* spp.
- *Batocera lineolata* infests boxelder, *Carya illinoensis*, *Fraxinus* spp., and *Quercus virginiana*.
- *Apriona rugicollis* infests *Malus x micromalus* 'American', *Cercis canadensis*, and *Gleditsia triacanthos*.
- The red neck (peach) longhorned beetle, RNLB, *Aromia bungii* occurred in most surveyed sites and caused serious damages to trees of *Prunus* such as peach and plum trees.
- *Psacotha hilaris* was found on mulberry trees; this beetle has already established in Italy and causes damage to fig trees.
- *Zeuzera coffeae* was found infesting quite a few *Malus x micromalus* 'American' trees in a nursery, causing break off of branches or dieback of stems. *Z. coffeae* larva reportedly feed on plants in more than 28 genera.
- *Ceresium sinicum*, which has been intercepted before and is on the Invasive Species List by Invasive.org, infests various species, such as red maple. Observed damages resulting from this pest included clean-cut twigs or stems of small nursery trees.
- *Acalolepta sublusca* was found on *Euonymus japonicus*, which is its primary host. The beetle also reportedly infested an herbal plant, *Tripterygium wilfordii*.

### Street, shade trees and trees in parks and botanical gardens in other provinces and municipalities

Major findings based on observations of 26 tree species:

- ALB is the primary woodborer infesting maple trees and different poplar tree species, especially in Northeast, Northwest, and North China.
- *Batocera lineolata* infest different trees such as *Eucalyptus robusta*, *Fraxinus velutina*, *Salix batylonica*, *S. matsudana*, and *Ligustrum lucidum* in quite a few provinces.
- In North China, ALB is the primary woodborer of *Platanus x acerifolia*, while in south China, its primary woodborer is CLB. In central China, both ALB and CLB attack this tree.
- EAB infested ash (*Fraxinus*) trees in North China.
- Willow trees such as *Salix batylonica* were infested by ALB, CLB, *Apriona rugicollis*, *Batocera lineolata*, *Megopis sinica*, and *Tremex* sp.
- Elm trees were attacked by both ALB and *Apriona rugicollis*, which also infest *Pyrus platycarpa* in central China.
- Black locust, *Robinia pseudoacacia*, was infested by *Tremex* sp.

## Conclusion

In summary, ALB, CLB, *Apriona rugicollis*, and *Batocera lineolata* are common pests in different areas of China. Infestation by these woodborers caused serious damage to North American host trees such as red maple, *Malus* spp, and others. The infestation level of RNLB, which primarily infest *Prunus* trees, has been on the rise in different areas in recent years. This beetle has been found in Japan as well as other countries. There were some infestations of North American trees in China by additional woodborers such as *Ceresium sinicum*, *Acalolepta sublusca*, *Zeuzera coffeae*, *Psacotha hilaris*, and *Tremex* sp. More studies are necessary to collect data on the distribution and host range of the woodborers found infesting North American trees in order to fully understand the risk the insects could pose if found in the U.S.

## Evaluation of potential host species for velvet longhorned beetle, *Trichoferus campestris*

David Cowan<sup>1</sup> and Baode Wang<sup>1</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

### Introduction

The velvet longhorned beetle, VLB, *Trichoferus campestris*, is an invasive wood boring beetle native to Asia and Russia that has been introduced into Europe and North America. In the United States, VLB is considered to be established in Illinois, Utah, and Wisconsin and has also been found in Colorado, Ohio, New Jersey, New York, and Pennsylvania [1] (Figure 1). Most of what is known about potential host species for VLB is based on identification of adults emerging from dry logs or wood products rather than from live, standing trees. Iwata and Yamada documented adult beetles that emerged from dry logs of beech, locust, mulberry, and walnut in Japan [2]. They concluded that an additional 36 genera of angiosperms and conifers could act as hosts though no additional evidence to support that assertion was provided. In 2011 VLB adults emerged and live larvae were extracted from a log taken from a dying Norway maple, *Acer platanoides*, in Mississauga, Ontario, Canada [3]. In 2014 multiple VLB larvae were found in cherry, hickory, and paper birch firewood collected from White Plains, New York and dissected at the Otis Laboratory. Over the following year, 22 adult VLB emerged from a portion of the wood that had not been dissected.



**Figure 1.** Velvet longhorned beetle specimen.

### Materials and Methods

Since 2014 researchers at the Otis Laboratory have been conducting visual surveys and installing wire-screen sleeve cages on potential host trees in order to catch emerging adult VLB. The primary field sites have been in orchards, city parks, and a golf course in the Salt Lake City vicinity where VLB is established. This work has demonstrated that stressed Russian olive, *Elaeagnus angustifolia*, and healthy and stressed sweet cherry, *Prunus avium*, and peach, *P. persica*, serve as hosts. This year, we continued to monitor 96 wire-screen sleeve cages that were installed in 2017 on sections of the following tree species: apple, *Malus pumila*, box elder, *Acer negundo*,

silver maple, *A. saccharinum*, river birch, *Betula nigra*, Russian olive, *Elaeagnus angustifolia*, honey locust, *Gleditsia triacanthos*, walnut, *Juglans* sp., white poplar, *Populus alba*, Fremont cottonwood, *P. fremontii*, sweet cherry, *Prunus avium*, cherry plum, *P. cerasifera*, peach, *P. persica*, black locust, *Robinia pseudoacacia*, Japanese pagoda, *Styphnolobium japonicum*, linden, *Tilia* sp., and elm, *Ulmus* sp. An additional 45 wire-screen sleeve cages were installed on specimens representing the species listed above, as well as on Austrian pine, *Pinus nigra*, Scots pine, *P. sylvestris*, Colorado blue spruce, *Picea pungens*, European filbert, *Corylus avellana* and western red cedar, *Thuja plicata*, in order to expand the number of potential host species being evaluated. From late May through mid-September the cages were checked on a weekly basis for beetle emergence.

In Bethel, Ohio, where VLB has been found, 22 wire-screen sleeve cages were installed on portions of tree species that had exhibited suspect VLB damage, including: black cherry, *Prunus serotina*, American beech, *Fagus grandifolia*, common hackberry, *Celtis occidentalis*, and sugar maple, *Acer saccharum*. These trees were located in wooded areas where adult VLB had been trapped the prior year. The cages were checked several times throughout the summer and early fall and monitoring will continue through next year. Otis Laboratory also provided VLB identification services to APHIS PPQ Field Operations in South Dakota. Black walnut, *Juglans nigra*, containing suspect VLB was sent to the lab for monitoring and dissection.

### Results

This year, a total of six adult beetles were caught in the wire-screen sleeve cages installed at an Orchard in Utah; two beetles from one apple tree and four beetles from two peach trees (Table 1).

**Table 1.** Number of velvet longhorned beetle caught in wire-screen sleeve cages by year and species.

Species	2014	2015	2017	2018
Apple	-	-	0	2
Cherry	5	13	0	0
Peach	4	14	2	4
Russian Olive	-	-	9	0

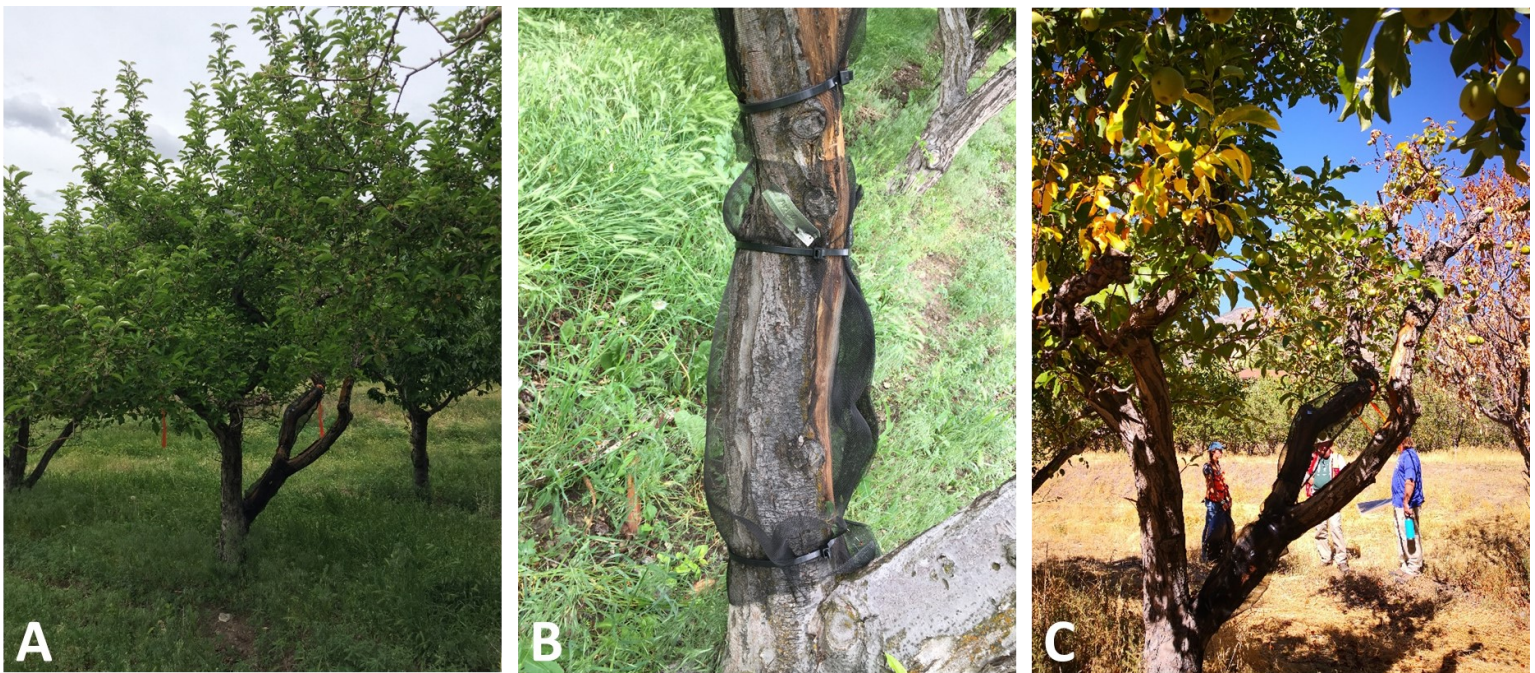


The identification of VLB from apple is the first confirmation that this species can serve as a host in the U.S. The tree full of foliage in May of 2018, though portions of bark were missing suggesting the tree was under physiological stress (Figure 2). In September the damaged limb appeared to be dying, although it had borne fruit, and the overall health of the tree had declined. The beetles caught on peach emerged from stressed and dead trees and are indicative that at least a portion of the population is taking more than one year to complete its life cycle as these cages were installed in 2017. This has also been observed in previous years in studies on cherry and peach. No VLB were caught in the wire-screen sleeve cages placed in Bethel, Ohio.

Larvae that were removed from wood from South Dakota were visually determined not to be *Trichoferus* sp. Subsequent molecular diagnostics confirmed the specimens to be rustic borer, *Xylotrechus colonus*, red-headed ash borer, *Neoclytus acuminatus*, and a Buprestid, *Chrysobothris sexsignata*. Five red-headed ash borer adults later emerged from the wood.

### Conclusions

- This work has demonstrated that live apple trees are acting as a host for velvet longhorned beetle in Utah.
- A portion of the velvet longhorned beetle population took more than one year to complete its life cycle.
- Suspect velvet longhorned beetle infested walnut from South Dakota did not contain VLB.



**Figure 2.** Apple tree on which two velvet longhorned beetles were caught in a wire-screen cage. A) Tree in May. B) Wire-screen cage. C) Tree in September.

### References

- 1) CAPS (Cooperative Agricultural Pest Survey) Program. Survey Status of Velvet longhorned beetle - *Trichoferus campestris*. 2013-2018 [online]. Available from <http://pest.ceris.purdue.edu/> [accessed 13 March 2018].
- 2) Iwata R, Yamada F. Notes on the biology of *Hesperophanes campestris* (Faldermann) (Col., Cerambycidae), a drywood borer in Japan. *Material und Organismen*. 1990; 25(4): 305-313.
- 3) Erin B-A, Troy K, Jean T. Discovery of *Trichoferus campestris* (Coleoptera: Cerambycidae) in Ontario, Canada and first host record in North America. *The Canadian Entomologist*. 2014; 146: 111-116.

## Asian longhorned beetle switch host feeding studies to evaluate host suitability

David Cowan<sup>1</sup> and Baode Wang<sup>1</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

### Introduction

The Asian Longhorned Beetle Eradication Program utilizes host removal and chemical treatment as methods of population control for Asian longhorned beetle, ALB, *Anaplophora glabripennis*, within quarantined areas. Host removal includes the elimination of known infested trees and may also include the removal of high risk host tree species in proximity to the infested trees [1]. Adult Asian longhorned beetles that remain after the removal of preferred and occasional hosts such as *Acer* spp., *Aesculus* spp., *Albizia* spp., *Betula* spp., *Cercidiphyllum* spp., *Fraxinus* spp., *Platanus* spp., *Populus* spp., *Salix* spp., *Sorbus* spp., and *Ulmus* spp. might attempt to utilize less preferred host species trees for feeding and reproduction. If less preferred host trees are capable of supporting beetle reproduction, this could prevent complete eradication of the population.

This series of studies evaluated the effect of changing adult ALB food source from a preferred host to an alternative host plant on adult longevity, feeding, fecundity and egg hatch. The results of these studies, as well as studies from prior years, provide information that enables APHIS PPQ to refine The Asian Longhorned Beetle: Annotated Host List [2], by contributing evidence about the suitability of a particular species as a host.

### Methods

Studies were conducted over an eleven month period. Beetles aged 15-30 days post emergence were selected, and placed as male-female pairs on potential host tree species.

Striped maple was used as a control. Experimental host tree species included apple, *Malus pumila*, balsam poplar, *Populus balsamifera*, blackgum, *Nyssa sylvatica*, London plane, *Platanus x acerfolia*, plum, *Prunus* sp. and sweetgum, *Liquidambar styraciflua*. In addition, one study included a negative control in which the beetles were given water but no food for the duration of the study.

Prior to the study, beetles were held individually in rearing containers and provided with striped maple twigs as food. During the study, paired beetles were held in mating containers with four twigs, 15 cm long by 0.2 – 0.7 cm in diameter, as a food source and provided with an artificial ovipositional substrate (cardboard 2.5 x 8.0 x 17.5 cm wrapped in multiple layers of cheese cloth). Study beetles were held under a 15 hour day/9 hour night (15L:9D) photoperiod, at 23°C and 55% RH. Beetle mortality, feeding, and oviposition were monitored.

The study was concluded when paired beetle mortality reached 100%. Eggs were removed from the mating containers and held in an incubator at 26° C for up to five weeks and monitored for hatching; larvae were not reared.

### Results

Survivorship of beetles fed on London plane was relatively good with at least half of the beetles living for 56 days whereas survivorship of beetles fed on sweetgum was as low as those given no food (Figure 1).

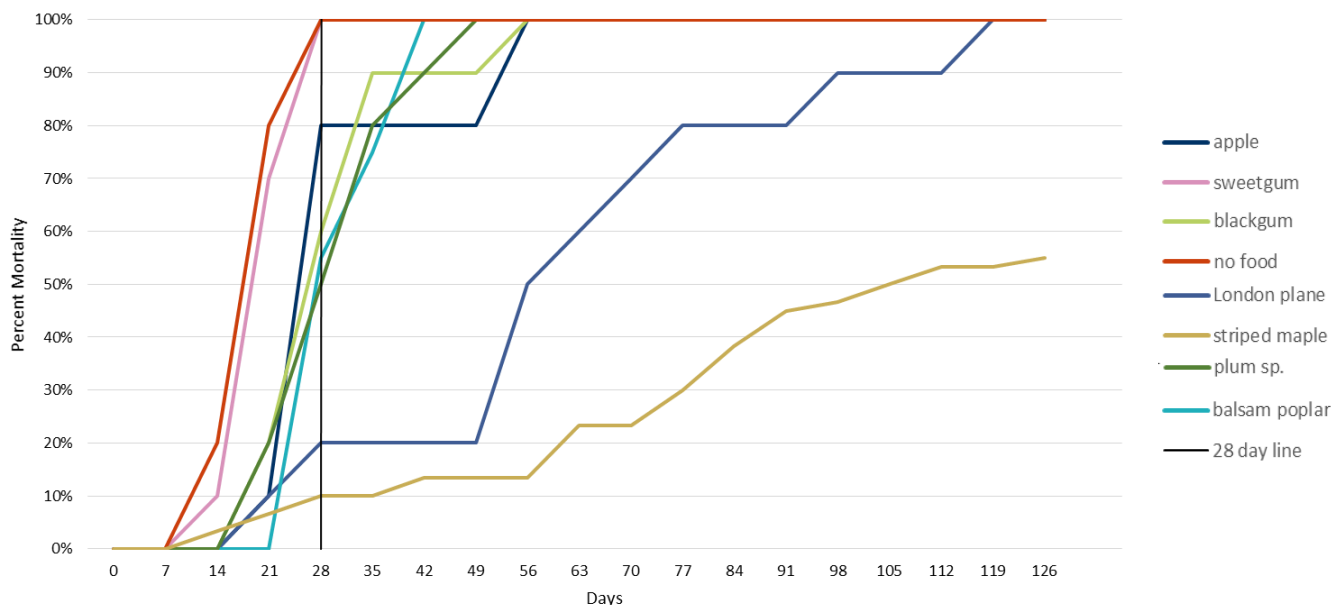
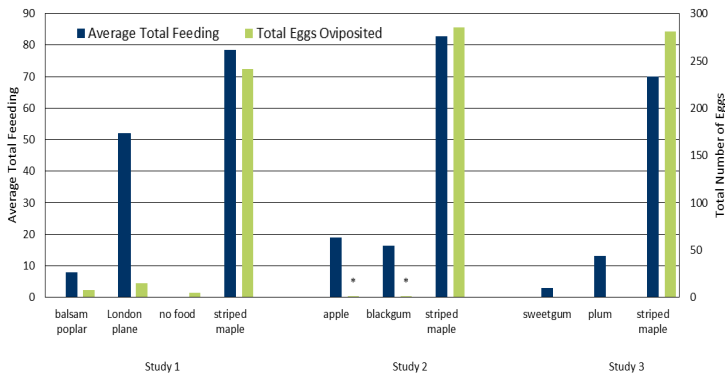


Figure 1. Adult ALB percent mortality by tree species out to 126 days.

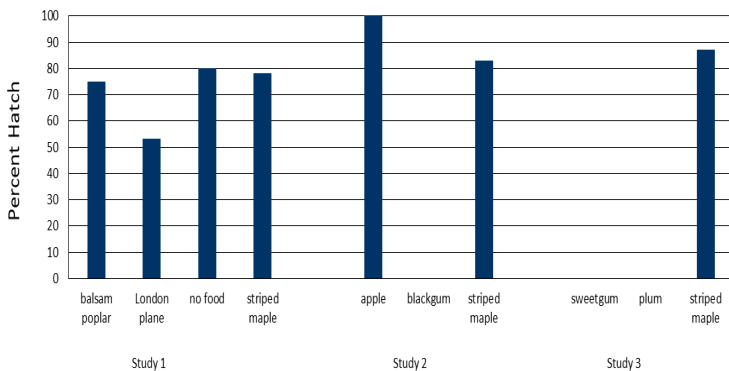
Survivorship of beetles fed on all other species was intermediate with 50 to 80 percent mortality seen by day 28. Female beetle lifespans were significantly reduced by switch host feeding on all species except plum. Male beetle lifespans were significantly decreased in all treatments.

Mated beetle pairs fed significantly less on all species of trees in the studies than on striped maple (data not shown). The average number of eggs oviposited by beetle pairs was significantly greater by those fed on striped maple in all studies (data not shown). Beetle pairs fed on sweetgum or plum did not produce any eggs. Beetle pairs fed on apple or blackgum produced one egg each, with only the apple egg being viable. Amount of feeding positively correlated with egg production in all studies (Figure 2).

Viable eggs were produced by mated beetle pairs fed on apple, balsam poplar, London plane and by those given no food, as evidenced by hatch rates (Figure 3).



**Figure 2.** Average total feeding and total eggs oviposited by mated beetle pairs by food source and study (striped maple = control). Asterisk indicates only one egg. Feeding as determined by a single observer using a scale from 0-10 to assess consumption of available food which was replaced as necessary over the lifespan of the beetles



**Figure 3.** Percent hatch by food source and study (striped maple = control).

**Conclusion**

Data from these studies indicate that the relative classifications of the tested species on the Asian Longhorned Beetle: Annotated Host List should remain unchanged. These studies have shown that:

- Male and female adults survived and fed on all host species evaluated in this study; however, ALB longevity was negatively impacted by switch host feeding
- Viable eggs were produced by mated beetle pairs fed on apple, balsam poplar and London plane, the number of oviposited eggs varied
- ALB fed on apple and blackgum oviposited one egg each, while ALB fed on sweetgum and plum oviposited no eggs
- Starved beetle pairs remained alive for several weeks and were able to oviposit viable eggs

Over the past four years, a total of 15 species and 21 genera have been assessed using the methodology presented here. The test species have included preferred, occasional to rare, and questionable, hosts as well as species that are unlisted. In general, these studies have demonstrated that switching adult ALB from a preferred host to a non-preferred host has detrimental, but not immediately devastating, effects on feeding, fecundity, and egg hatch. Further studies are warranted in order to determine how switch host feeding on live trees, rather than twigs, would affect those measures. The ALB Eradication Program should remain cognizant of the fact that non-preferred host species may provide sufficient resources for completion of the ALB life cycle.

**References**

- 1) Rose, Robyn. Asian Longhorned Beetle Response Guidelines. [Online]. Available from: [https://www.aphis.usda.gov/plant\\_health/plant\\_pest\\_info/asian\\_lhb/downloads/response-guidelines.pdf](https://www.aphis.usda.gov/plant_health/plant_pest_info/asian_lhb/downloads/response-guidelines.pdf) [April 4, 2019]
- 2) Wang, B. Asian Longhorned Beetle: Annotated Host. [Online]. Available from: [https://www.aphis.usda.gov/plant\\_health/plant\\_pest\\_info/asian\\_lhb/downloads/hostlist.pdf](https://www.aphis.usda.gov/plant_health/plant_pest_info/asian_lhb/downloads/hostlist.pdf) [April 4, 2019]



## Gypsy moth production activities in 2018

Hannah Nadel<sup>1</sup>, Hannah Landers<sup>1</sup>, Susan Lane<sup>1</sup>, Chris McCallum<sup>1</sup>, Marci Murray<sup>1,2</sup>, and Lara Trozzo<sup>1</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>University of Massachusetts Amherst, Department of Environmental Conservation, Amherst, MA

### Introduction

The New Jersey strain of European gypsy moth, EGM, *Lymantria dispar dispar*, has been maintained in colony at the Otis Laboratory for 76 generations. It produces life stages for both colony maintenance and moth production to fill the needs of domestic and foreign customers and cooperators. A new colony cohort is established weekly, producing about 1,000 egg masses. The mass-reared colony, maintained by a staff of three technicians and a project leader, fulfills needs for research, training, education, and outreach.

Six colonies of related gypsy moths were maintained in the Otis Insect Containment Facility. These consist of four strains of Asian gypsy moth, AGM, *Lymantria dispar asiatica*, from China, Korea, Mongolia, and central Russia, a separate subspecies from Japan, *Lymantria dispar japonica*, and a related species, rosy moth, *Lymantria mathura*. Past and future applications for these colonies include research to reduce risk of AGM entry into the country, development of trap technology and molecular diagnostics for enhanced detection and identification, and phenological studies for mapping areas at risk of establishment.

### Production Activities

The EGM colony served several purposes in 2018 (Table 1). Over 50,000 egg masses were produced, many of which were cycled back into the colony or sent to researchers in the United States, Canada, and Europe. An excess of eggs is produced so that eggs can be mixed to retain genetic diversity. Eggs and other EGM

life stages were distributed to 14 U.S. institutions and four foreign institutions. Over 128,000 male pupae were reared for projects affiliated with the Gypsy Moth Slow the Spread Foundation, to conduct research on mating disruption and efficacy of mating-disruption products. Egg masses were provided to a co-operator in Minnesota to determine the dispersal capacity of EGM larvae and assess if host-free buffer zones are effective at preventing spread from lumber piles across a zone free of host plants. Substerile EGM egg masses, which are able to hatch but develop into non-breeding adults, were provided to Washington State Department of Agriculture to monitor egg-hatch dates in the field. Hatch dates, combined with weather data, enable the state to estimate when to place traps for adult moths in summer at sites where trapped AGM may have bred in 2018; trapped moths in the summer of 2019 would confirm breeding and may trigger eradication efforts. Other egg masses were regularly sent to the USDA Agricultural Research Service Laboratory in Beltsville, MD for research on EGM pathogens. Regular shipments of egg masses are sent to Europe for research on insects that parasitize EGM, as well as on EGM ecology and effects on plants. Lastly, many specimens and life-cycle displays were provided to PPQ outreach and training programs in the U.S. The AGM colonies were utilized only for outreach and training in 2018; specimens were used to create comparative displays of AGM and EGM for use by state pest surveyors and at outreach events.

**Table 1.** Purpose and number of EGM life stages and specimens reared and provided in 2018 to U.S. and foreign institutions.

Purpose	Egg Masses	Larvae	Pupae ♂	Pupae ♀	Riker Mounts	Specimens
Research: GM microbial interactions	1,150					
Research: GM biocontrol	1,325					
Research: host resistance, host-plant interactions	1,292	2,000				
Outreach, training, and education	615				54	225
Research & control: Slow the Spread Foundation	183		128,400			
Research: GM ecology	85					
Estimate egg-hatch date in AGM-detection site in WA	15					
Research: larval dispersal capacity	100					
Research: unspecified	800					
Colony maintenance	10,400	156,000	78,000	78,000		
<b>Total</b>	<b>15,965</b>	<b>158,000</b>	<b>206,400</b>	<b>78,000</b>	<b>54</b>	<b>225</b>

## Moth research colonies: Rearing methods and projects supported

Hannah Nadel<sup>1</sup>, Lara Trozzo<sup>1</sup>, Hannah Landers<sup>1</sup>, Sue Lane<sup>1</sup>, and Marci Murray<sup>1,2</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>University of Massachusetts Amherst, Department of Environmental Conservation, Amherst, MA

### Introduction

Four moth colonies were reared in 2018 for research:

1) corn earworm, CEW, *Helicoverpa zea*, 2) Old world bollworm, OWB, *Helicoverpa armigera*, 3) European grapevine moth, EGVM, *Lobesia botrana*, and 4) navel orangeworm, NOW, *Amyelois transitella*. All but CEW were held in the Otis Insect Containment Facility. Both OWB and CEW are destructive to many important crops including corn, cotton, tomatoes, soybean, and tobacco. Corn earworm is native and widespread throughout the United States, but OWB is not yet present in the continental U.S. It is established in Puerto Rico and has been detected in traps baited with CEW pheromone [1]. Trapping surveys for OWB are hampered by the morphological similarity between adult OWB and CEW, as well as the lack of a specific lure for OWB. Therefore, colonies of both species are being reared to support research on specific lure development and on molecular and visual methods to distinguish between the two species and their hybrids. We continued to maintain a EGVM colony despite it being eradicated from the U.S. in 2016, to validate irradiation treatment of pupae in the event that sterile EGVM releases become necessary and to provide color-marked males for pest-survey training in California. Lastly, we reared an Otis Lab colony and received experimental NOW from the APHIS NOW Rearing Facility in Phoenix, Arizona to assess male quality for sterile release after irradiation and handling treatments. Sterile-male releases to control damage by this native pest in nut crops are being tested in California.

### Old world bollworm and corn earworm

Rearing protocols for OWB were modified in 2018 after a strong decline in the colony due to unknown causes. Pathogen tests discounted most of the possible disease agents that could cause such declines except viruses, which will be evaluated in 2019. The source colony in Spain suffered a similar decline. Fortunately, the colony began to recover with increased sanitation and by rearing larvae in individual cells rather than in groups. A study comparing survival and weight of moths reared on Frontier Scientific Lepidoptera Diet (Newark, Delaware) and the standard in-house gypsy moth diet showed that survival was not affected by diet, while weight was higher on the gypsy moth diet. Rearing protocols for CEW have not changed since 2017.

Research, conducted at the Fort Collins Laboratory, on both colonies was focused on distinguishing between the two closely-related species and their hybrids (Table 1).

All ages of OWB larvae were used to test a squash-blot and dot-blot ELISA assay developed to differentiate between OWB and CEW. These results are being used to synthesize a new antibody that is more specific to target proteins. Hybrids of CEW and OWB were produced after nine group-caging attempts and were utilized to evaluate the performance of existing molecular assays to differentiate between these two species. The data validated prior results for wild-caught specimens that were assumed to be hybrids and provided a baseline for assay performance when hybrids are analyzed. Hybrids were also compared with those from the Puerto Rico colony using morphometrics to determine if there was any difference in first-generation offspring that could be correlated with cross direction (mothers of PR hybrids were CEW, while mothers of Otis hybrids were OWB).

### European grapevine moth

The colony has been maintained since 2010. In 2018 experimental insects were provided to validate efficacy of an optimal dose (~ 140 Gy) of irradiation to sterilize mature pupae (Table 1). The efficacy of this treatment was tracked for two generations to ensure low fertility of offspring from irradiated parents. The treatment may be used as an option for sterile-insect release programs. The colony also annually provides color-marked males for training survey trappers in California. The moth is reared on artificial diet containing calco-red dye and the color remains in the tissues after death. This enables the pest-survey program to determine if trapped moths were lab-reared and “planted” in traps to test trapper ability to recognize EGVM, or if the moths are unmarked wild individuals requiring emergency response.

### Navel orangeworm

The colony was sourced in early 2016 from the NOW rearing facility in Phoenix, AZ and was reared on diet prepared at the facility for mass-rearing development and sterile-insect production. Otis moths and moths shipped from Phoenix were studied in cages to determine effects of irradiation, adult collection, and shipment by airline on flightability of adult males. Flightability tested both male ability and proportion flying out of a cylinder. Exposure to 300 Gy irradiation treatment in Phoenix did not significantly affect male flightability, but adult collection and air shipments were somewhat deleterious. However, the latter results were too variable to draw robust conclusions.

**Table 1.** Numbers and purposes of old world bollworm, OWB, corn earworm, CEW, European grapevine moth, EGVM, and navel orange-worm, NOW, life stages and specimens provided in 2018.

Species	Purpose	Eggs	Larvae	Adult ♂	Adult ♀	Hybrids (OWB♀ x CEW♂)	Specimens
OWB	Morphological diagnostics of foreign and native heliothine eggs	100					
	Morphological diagnostics of all life stages	20	50	15	15		
	ELISA assay development to distinguish larvae from CEW and hybrids		20				
	Genotyping, development of diagnostic assays, and morphometric comparison with hybrids from CEW mothers				15	12	
	Outreach and training displays						120
CEW	Morphological diagnostics of foreign and native heliothine eggs	100					
	ELISA assay development to diagnose larvae		20				
	Genotyping and development of diagnostic assays vs. OWB & hybrids			15			
EGVM	Internally red-marked males for pest-survey training in CA						250
	Validation of irradiation treatment for sterile insect technique	12,145		650	650		
NOW	Assays assessing effects of irradiation and handling on sterile-male quality		460	1,840			

## References

- 1) Old World Bollworm [Internet]. USDA APHIS; c2018 [cited 2019 March 28]. Hungry Pests. Available from: <https://www.aphis.usda.gov/aphis/resources/pests-diseases/hungry-pests/the-threat/old-world-bollworm/owb>.

## Longhorned beetle production activities in 2018

Hannah Nadel<sup>1</sup>, Carrie Crook<sup>1</sup>, Tanya Cameron<sup>1</sup>, Sam Stella<sup>1,2</sup>, and Marci Murray<sup>1,3</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>Xavier University, Biology Department, Cincinnati, OH

<sup>3</sup>University of Massachusetts Amherst, Department of Environmental Conservation, Amherst, MA

### Introduction

Five colonies of Asian longhorned beetle, ALB, *Anoplophora glabripennis*, are mass-reared in the Otis Insect Containment Facility (OICF). The colonies originated from collections in China, Ohio, Massachusetts, and New York. A mixed colony from the four strains is also reared. These colonies support research and development on detection, control, and eradication of the pest. Weekly production schedules, rearing methods on artificial diet, and tracking are described in the 2016 Otis Annual Report.

Small colonies of two other longhorned beetles from China are maintained in the OICF: Chinese mulberry beetle, CMB, *Apriona rugicollis* (= *A. germari*) and velvet longhorned beetle, VLB, *Trichoferus campestris*. Chinese mulberry beetle has not been recorded in North America but has been found in wood packaging arriving in Europe from China, and preemptive steps should be taken in the event that it enters the United States. Velvet longhorned beetle is frequently found in wood packaging with trade goods from China and is breeding in at least three states in the U.S. Both colonies will be used to determine which tree species are at risk for damage and the seasonal temperatures the insects require to complete their life cycle.

### Asian longhorned beetle

Research, public outreach, and training programs were supported by the ALB colonies in 2018 (Table 1). Details of supported research projects are as follows:

- Chemical ecology: A method was developed to rear ALB from egg to adult in several rooted species of willow within the OICF. Volatiles produced by adults reared in living host plants are often produced in higher amounts and quality compared to those produced by insects feeding on artificial diet.
- Chemical ecology: 20-24 ALB adults were isolated and maintained weekly for enhanced pheromone research using broad-spectrum lighting to induce production of greater quantities of pheromone.

- Host-range testing support and fecundity studies: ALB life stages and plant material were provided for an ongoing study.
- Biological control support: ALB life stages were provided for studies conducted in the OICF on parasitic insects imported from Asia; supported studies included use of ALB larvae in “sentinel logs” to establish environmental conditions and cage design for future field studies.
- Acoustic-detection support: Insects and wood material were provided for Stevens Institute of Technology engineers to develop an acoustic system and algorithm for detection of wood-boring insects in wood packaging at ports and in other applications.
- Colony health: ALB larvae were examined for possible pathogens, and sanitation protocols were refined to prevent contamination.
- Outreach and training: Hundreds of adult specimens were preserved; adult and larval specimens were provided for outreach and training displays.

### Velvet longhorned beetle

A small-scale study, which remains in progress, suggests that VLB larvae reared on artificial diet pupate at significantly higher rates when exposed to cool, winter conditions at maturity than when maintained at a constant 23°C. Winter diapause in larvae was induced by exposure of mature larvae to fluctuating daily temperatures simulating fall conditions in a temperate climate for one week. Subsequent chilling of larvae at 10°C for 3-4 months, followed by returning larvae to 23°C, is showing promise for pupation and completion of the life cycle. Results for chilling at 5°C are pending.

### Chinese mulberry beetle

A moderate-sized colony was maintained on artificial diet without winter chill for several generations for the Pest Management Lab. Survival of eggs and newly-hatched larvae was improved by leaving them in mulberry twigs where the female deposited the eggs, and transferring them to artificial diet after a few weeks.

**Table 1.** Number of Asian longhorned beetle life stages produced in 2018, and specimens provided to research.

Purpose	Eggs	Neonates	Older Larvae	Pupae	Adult ♂	Adult ♀
Training and outreach	180	80	150	80	310	280
Research: chemical ecology					168	218
Rearing development in willow			21		30	30
Pathogen study			40		10	10
Research: biocontrol					100	100
Research: host-range					40	40
Colony maintenance	4,966	3,484	2,854	2,356	517	408
<b>Total</b>	<b>5,146</b>	<b>3,564</b>	<b>3,065</b>	<b>2,436</b>	<b>1,175</b>	<b>1,086</b>

## Host suitability studies for spotted lanternfly

Miriam Cooperband<sup>1</sup>, Kelley Murman<sup>1,2</sup>, Stefani Cannon<sup>1,2</sup>, Leslie Abreu<sup>1,2</sup>, Longwa Zhang<sup>3</sup>, Matthew Wallace<sup>2</sup>, Jacob Wickham<sup>4</sup>, and Emelie Swackhamer<sup>5</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>East Stroudsburg University, Department of Biological Sciences, East Stroudsburg, PA

<sup>3</sup>Anhui Agricultural University, School of Forestry, Hefei, China

<sup>4</sup>Chinese Academy of Sciences, Institute of Chemistry, Beijing, China

<sup>5</sup>Pennsylvania State University, Montgomery County Extension Office, University Park, PA

### Introduction

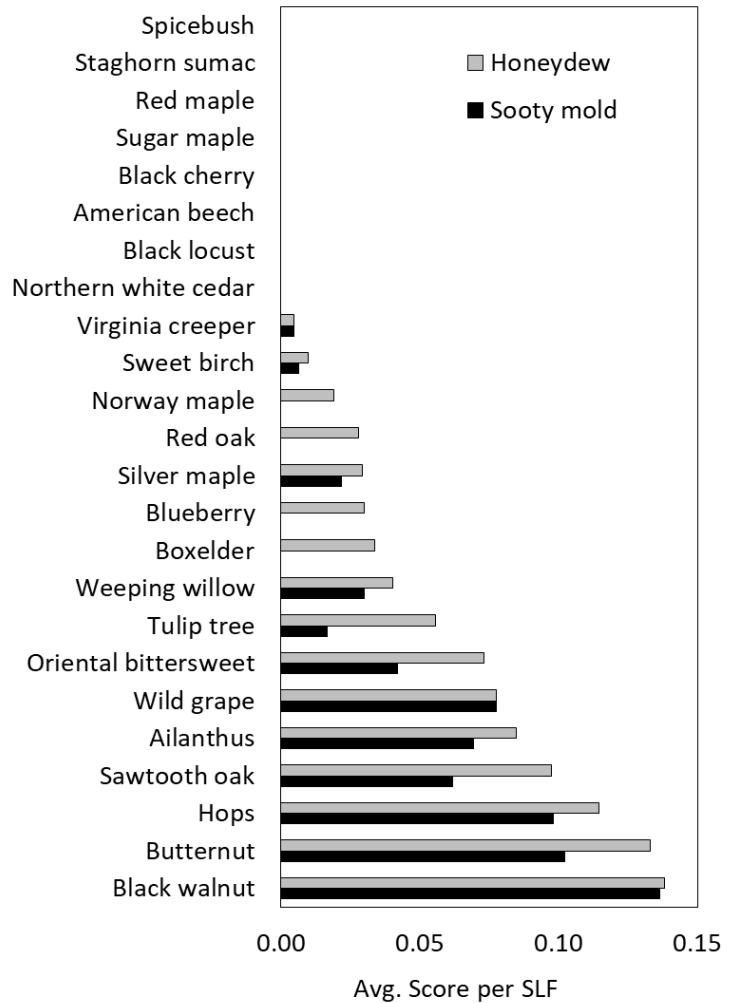
The spotted lanternfly, SLF, *Lycorma delicatula*, is a newly invasive pest native to China which was discovered in Pennsylvania in 2014. It has now spread to 15 Pennsylvania counties and to Virginia, and has been detected in several other neighboring states (PA, VA, NJ, DE, CT, NY, MA). SLF have been observed to feed on a broad range of over 70 host plants, but we have found some host plants are more suitable for feeding, survival, and development than others. Their preferred host, tree-of-heaven, *Ailanthus altissima*, is also invasive from China. Host removal may be a useful control strategy if alternative suitable hosts are unavailable or not preferred. This study examined numerous host plant species to evaluate the degree to which SLF can feed, survive, and develop on species other than *A. altissima*.

### Methods

Host suitability studies were mostly conducted in the field in Pennsylvania, with one host species tested in China, and the preferred host, *A. altissima*, was tested in both locations as a control. Each test consisted of a sleeve on a branch in which 10 first instar spotted lanternfly nymphs were placed at the beginning of the summer. Sleeves were monitored weekly for: feeding evidence such as honeydew and sooty mold by assigning scores to level observed, number surviving, and stages reached. In this manner, 25 plant species were tested in 2017 and 2018.

### Conclusions

We found that not all hosts are equally suitable for feeding (Figure 1), survival (Figure 2), and development (Figure 3) of spotted lanternfly.



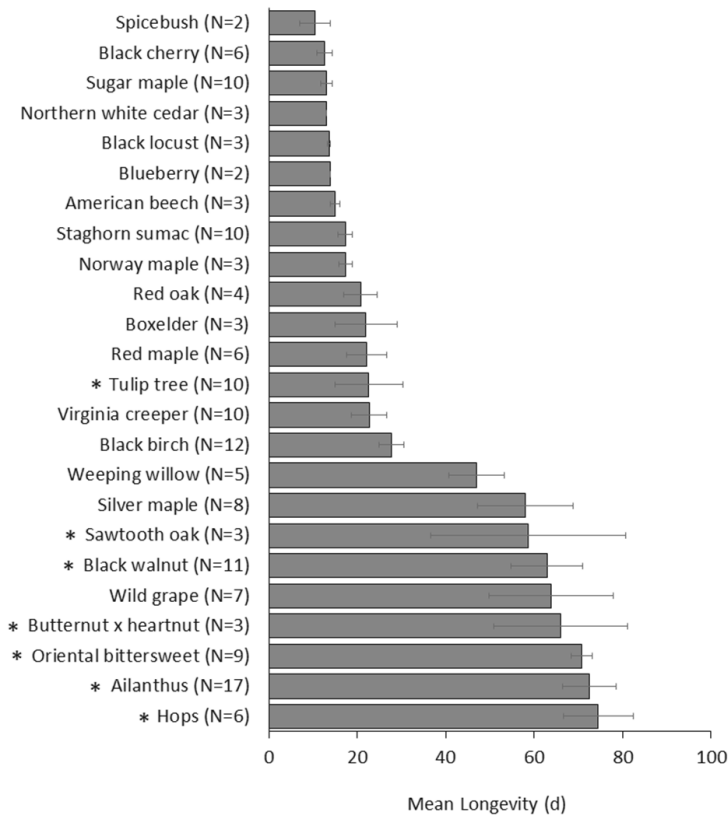
**Figure 1.** Evidence of feeding indicated by the presence of honeydew and sooty mold. Scores corrected for number of SLF alive in each sleeve.



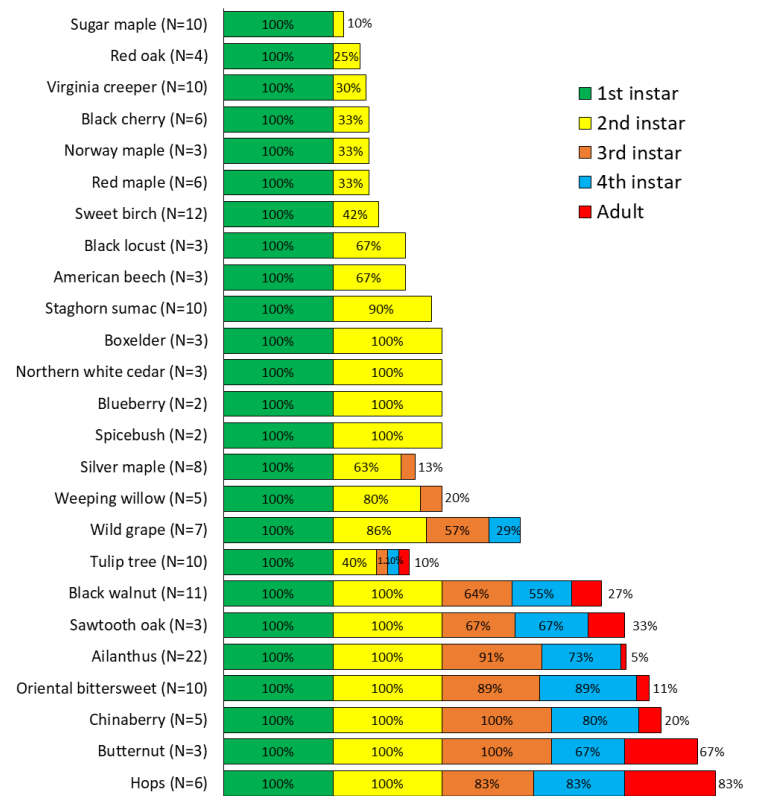
In addition to *A. altissima*, we identified three hosts in 2017 and four hosts in 2018 that were capable of supporting spotted lanternfly development from first instar to adult: hops, butternut, chinaberry, oriental bittersweet, sawtooth oak, black walnut, and tulip tree. Tulip tree was unusual in that out of 100 first instar nymphs, only one survived past the second instar and made it to adulthood. In our study, grape was able to support spotted lanternfly survival to fourth instar, but they did not develop to adults.

All adults died within a week or two after eclosion due to difficulties keeping SLF alive in captivity. Therefore, we were unable to determine how long adults could feed and survive on those hosts, or whether they could later oviposit and produce viable eggs.

Some species that are anecdotally very attractive in the field such as spicebush, red maple, and staghorn sumac, did not prove to be suitable hosts in this study. Although numerous plants may be suitable for nymphs to feed and survive for a short time, nutritional requirements for successful development and advancement to other stages are not necessarily met by those hosts. Only eleven of the 25 species tested produced third instars and moreover only eight species produced adults.



**Figure 2.** Longevity in sleeves starting with first instar SLF. Mean number of days that (N) sleeves contained living SLF. Asterisk indicates species that produced at least one adult.



**Figure 3.** Percent of sleeves reaching developmental stages starting with 1st instar SLF (PA & China combined). Mean percent of field sleeves on different host plants, in which first instar SLF developed to subsequent stages. Tests were conducted in Pennsylvania except for five sleeves on *Ailanthus* and five on chinaberry, which were tested in China. N represents the number of sleeves (replicates) that started with 10 first instar nymphs on each host plant species.

## Host plant volatiles that elicit antennal responses and attraction in spotted lanternfly

Miriam Cooperband<sup>1</sup>, Nathan Derstine<sup>1,2</sup>, Linnea Meier<sup>1,2</sup>, Isaiah Canlas<sup>1,2</sup>, and Daniel Carrillo<sup>2</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>University of Florida, Tropical Research and Education Center, Gainesville, FL

### Introduction

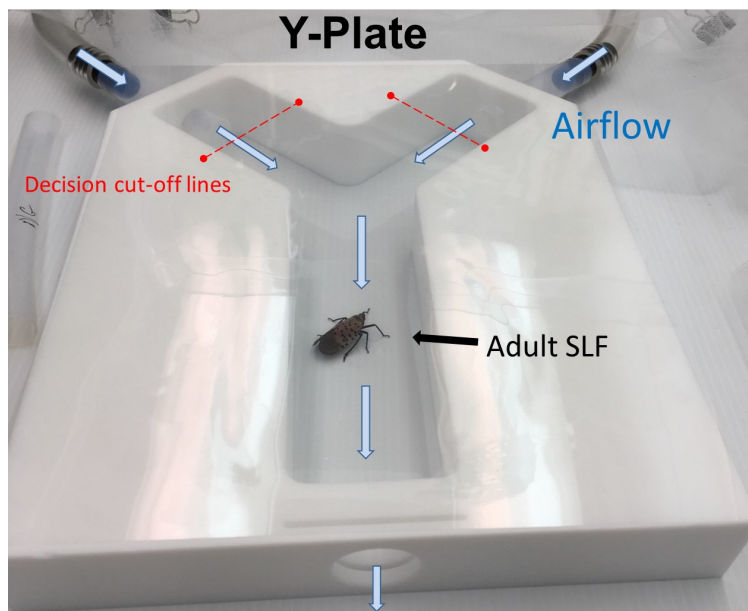
The spotted lanternfly, SLF, *Lycorma delicatula*, is an invasive phloem feeder from China with a broad host range. Its primary host is tree of heaven, *Ailanthus altissima*, which originates from China and was brought to the United States in 1784 and has since become a ubiquitous weed tree. Notably, *L. delicatula* feeds on, damages, and even kills economically important plants such as grape, walnut, and hops. It is also found in large numbers feeding or ovipositing on apple, oak, maple, willow, cherry, and over 70 other species. It poses a serious threat to the grape and timber industries, and is also a nuisance pest in urban and suburban areas. Its cryptic egg masses are deposited on any surface or crevice, creating a high risk of spread through human assisted movement. Our previous work resulted in the development of a kairomone (host plant volatile) lure containing methyl salicylate that is currently the best known attractant for SLF. Although the lure can enhance trap capture under certain circumstances, better lures are still desperately needed. To improve our understanding of kairomones used by SLF, we investigated the antennal and behavioral responses of SLF to volatiles produced from several host plants and compared them to their preferred host, *A. altissima*.

### Methods and Results

We collected volatiles from eleven different host plant species that were considered strongly attractive based on field observations. Gas chromatography coupled with electroantennographic detection (GC-EAD) was used to screen volatiles for antennal responses. Antennal responses indicated that SLF could detect specific individual compounds with their antennae. From these eleven species of host plants, we found roughly 37 unique compounds that elicited antennal responses, and have chemically identified 17. These compounds were then purchased, synthesized, or isolated for further testing.

Although GC-EAD can tell us which compounds SLF are capable of detecting with their antennae, it cannot tell us what type of behavioral response, if any, these compounds elicit. Therefore, these 17 antennally active compounds were tested for attraction in a dual choice Y-plate olfactometer, along with three other compounds of interest (Figure 1). The behavioral bioassay can indicate whether volatiles being tested are attractive, repellent, or neutral to SLF. The 17 antennally active compounds associated with SLF host plants, when tested individually, were all found to be either significantly attractive or trending attractive in the Y-plate bioassay (Table 1).

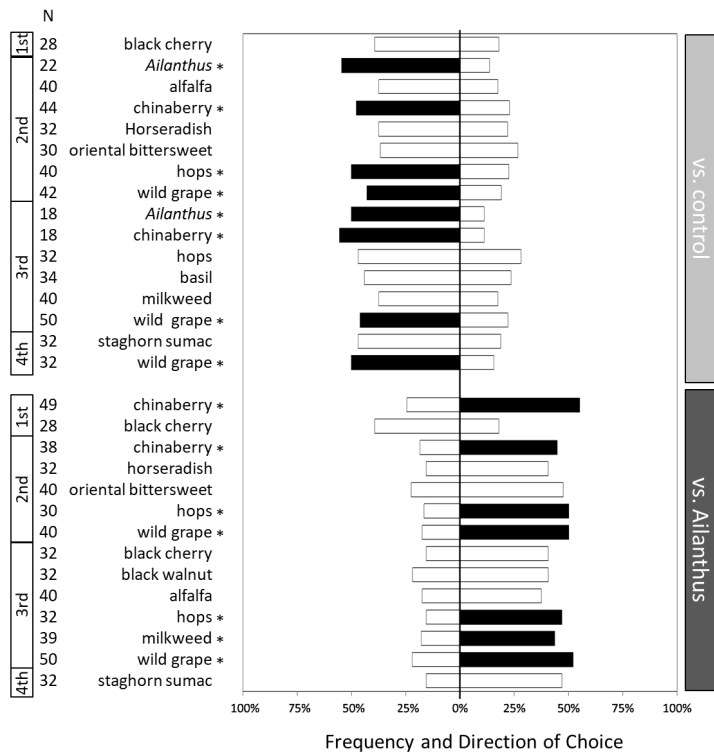
The dual choice olfactometer was also used to measure attraction to odors emitted from living host plant material and compared to either blank controls or volatiles from their preferred host, *A. altissima*. Nearly all plants tested were either significantly attractive or trended slightly attractive when compared to blank controls.



**Figure 1.** Dual choice Y-plate olfactometer being used to compare SLF responses to volatiles.

However, when compared to *Ailanthus* volatiles, SLF was nearly always significantly more attracted to or trending towards *Ailanthus* volatiles over volatiles from the competing plant (Figure 2). Sulcatone is a compound that was found in certain volatile collections of *A. altissima* in 2018. Interestingly, sulcatone was the only kairomone that attracted a larger percent (trend) of SLF when tested against methyl salicylate. This compound was formulated into lures and field tested in 2018 in both China and Pennsylvania. A significant dose response to sulcatone was demonstrated in these field studies.

SLF were significantly attracted to *Ailanthus*, chinaberry, wild grape, and hops when compared to a blank control. However, when comparing plants against *Ailanthus* volatiles, SLF significantly preferred volatiles from *Ailanthus* over chinaberry, wild grape, hops, and milkweed (Figure 2). Although not significant, all other plants tested showed an attractive trend when compared to a blank control, but SLF trended towards *Ailanthus* over these other plants with the exception of first instars trending toward black cherry (Figure 2).



**Figure 2.** Frequency and direction of choice of SLF nymphs tested in a dual choice olfactometer walking bioassay. Nymphs were offered a choice between volatiles from various host plants (left) and either blank controls or volatiles from *Ailanthus* (right). Asterisks and black bars indicate a significant preference of one side over the other (Chi-square test,  $\alpha=0.05$ ).

Volatile profiles of 10 additional commonly attacked host plant species were examined for the 15 antennally active and attractive compounds found in *A. altissima* volatile profiles. These other host plants shared between four and 10 of the same active compounds (Figure 3). Interestingly, all of them had methyl salicylate in common.

**Conclusions**

By using GC-EAD, a technique to record spotted lanternfly antennal electrophysiological responses, we identified 15 kairomones, many of which are found in other hosts that SLF tends to attack. This illustrates the polyphagous nature of spotted lanternfly. Of these, only methyl salicylate is found in the volatile profiles of all 11 host plants we examined. Sulcatone was the only compound that trended more attractive than methyl salicylate for some stages. Out of the 11 species examined, sulcatone was found in *A. altissima*, black cherry, and spicebush. Lures containing sulcatone were field tested, demonstrating a significant dose response to this kairomone. Future work in 2019 will include comparing lures with sulcatone and methyl salicylate.

15 ACTIVE COMPOUNDS	<i>Ailanthus</i>	Hops	Black Cherry	Black Walnut	Virginia Creeper	Spicebush	Grape	Chinaberry	Staghorn Sumac	Oriental Bittersweet	Red Oak	TOTAL
methyl salicylate	x	x	x	x	x	x	x	x	x	x	x	11
(E,E)- $\alpha$ -farnesene	x	x	x	x	x		x	x	x	x	x	10
$\beta$ -ocimene	x	x	x	x	x	x		x	x	x		10
(E)-4,8-dimethyl-1,3,7-nonatriene	x	x	x	x	x	x	x				x	9
$\beta$ -caryophyllene	x	x	x	x	x	x			x	x		9
$\beta$ -ylangene	x	x		x	x	x			x	x		8
(Z)-3-hexenyl acetate	x	x		x	x			x				5
linalool	x		x		x	x		x				5
(Z)-3-hexenol	x	x	x	x								4
geranylacetone	x		x					x	x			4
sulcatone	x		x			x						3
4-terpineol	x				x							2
1-octen-3-ol	x	x										2
methyl benzoate	x						x					2
(Z)-3-hexenyl butyrate	x	x										2
<b>TOTAL</b>	<b>15</b>	<b>10</b>	<b>9</b>	<b>9</b>	<b>8</b>	<b>7</b>	<b>7</b>	<b>6</b>	<b>6</b>	<b>5</b>	<b>4</b>	

**Figure 3.** Fifteen antennally active host plant volatiles that were found to be attractive, or enhance attraction, to SLF (kairomones), and their presence or absence in 11 host plant species on which SLF are frequently found.

**Table 1.** A summary of 20 volatile compounds tested in the dual choice olfactometer, comparing SLF preferences between the compound tested and either a blank control or methyl salicylate. Plus signs (+) indicate the compound tested was chosen significantly more than the alternative, whereas minus signs (-) indicate the alternative was chosen significantly more than the compound tested. Tests that were not significant (n.s.) and showed trends with at least 15% difference between choices towards (tr+) or away from (tr-) the compound tested are shaded light green or light blue, respectively, or otherwise were left unshaded. The first 15 compounds are those found to be antennally active and present in *Ailanthus* volatile collections. Compounds 16 and 17 were found in black walnut and produced uncharacteristic antennal responses, and the last three compounds were tested for other reasons.

	Compound tested	Versus control						Versus Methyl salicylate					
		1st	2nd	3rd	4th	females	males	1st	2nd	3rd	4th	females	males
1	methyl salicylate	++		++	++	++	tr+						
2	methyl benzoate			tr+	++			--				tr-	
3	Z-3-Hexenyl Butyrate			tr+							tr-		
4	Z-3-hexenol	tr+		tr+	++	++						tr-	
5	1-octen-3-ol			++	tr+						tr-		
6	4-terpineol	++	++	tr+				-- <sup>a</sup>				tr-	
7	Linalool (racemic)	++						tr- <sup>a</sup>					
8	(E,E)- $\alpha$ -farnesene	n.s.		++	++	++						n.s.	
9	$\beta$ -caryophyllene	++		tr+						tr-			
10	$\beta$ -ylangene				++								
11	4,8-dimethyl-(3E)-1,3,7-nonatriene			tr+		tr+					--		
12	$\beta$ -ocimene isomer mix	tr+	tr+			n.s.							
13	sulcatone			++		tr+	++			tr+	tr+		
14	geranylacetone				tr+						--		
15	Z-3-hexenyl acetate				tr+								
16	$\alpha$ -pinene	n.s.						--					
17	$\beta$ -pinene			tr+									
18	cantharidin					tr+							
19	3-carene				n.s.	n.s.							
20	(E)- $\beta$ -farnesene		n.s.										

<sup>a</sup> All treatments used 1 mg of material except for these which compared 1 mg of compound against 2 mg of methyl salicylate.



## Phenological studies on spotted lanternfly host plants, adult biology, and chemical ecology

Miriam Cooperband<sup>1</sup>, Linnea Meier<sup>1,2</sup>, Isaiah Canlas<sup>1,3</sup>, Kelley Murman<sup>1,4</sup>, Stefani Cannon<sup>1,4</sup>, Sam Stella<sup>1,3</sup>, Chris Smith<sup>1,2</sup>, Leslie Abreu<sup>1,4</sup>, Matthew Wallace<sup>4</sup>, Daniel Carrillo<sup>2</sup>, and Ann Ray<sup>3</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>University of Florida, Tropical Research and Education Center, Gainesville, FL

<sup>3</sup>Xavier University, Biology Department, Cincinnati, OH

<sup>4</sup>East Stroudsburg University, Department of Biological Sciences, East Stroudsburg, PA

### Introduction

The spotted lanternfly, SLF, *Lycorma delicatula*, is a newly invasive pest discovered in Pennsylvania in 2014, which is native to China. This species has a broad range of over 70 host plants, but its preferred host, tree-of-heaven, *Ailanthus altissima*, is also invasive from China and broadly distributed. Although it has been found on numerous species of plants at various times, it appears to accumulate on its preferred host *A. altissima* at certain times. While adults are present in Pennsylvania from the end of July until early November, very little is known about their reproductive biology and phenology, or how, where, and when they find each other to mate. A number of studies were conducted in 2018 to shed light on these important questions.

### Methods

#### Dissections

On a weekly basis from August 1 until October 22, ten adult males and females were field collected, measured, weighed, and dissected to determine mating status and egg load. At the same time in the field, we recorded dates of first observed mating, period of mass flight, and first observed egg mass.

#### Distribution among host plants

Five transects in forested and rural residential areas were selected, each with a cluster of *A. altissima* trees at one end. All trees (>15 cm DBH) along the transects were banded and monitored biweekly from May (1<sup>st</sup> instar emergence) until October (ovipositing adults).

#### Sex ratio shift

Field trapping of adults provided data on when sex ratios on tree trunks shifted from being roughly 50:50 to being highly female-biased.

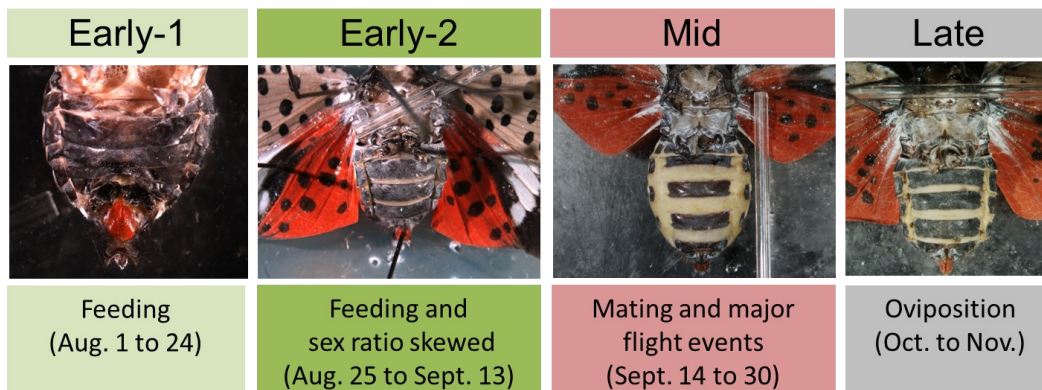
#### Chemical ecology of adults

Volatile collections and body washes were collected from males and females on a weekly basis for 12 weeks starting the first week in August. Body washes were tested for attraction against male and female SLF in laboratory Y-plate bioassays. Volatile collections were analyzed using gas chromatography coupled with electro-antennographic detection (GC-EAD) in attempts to identify sex-specific antennally active compounds that may be responsible for attraction.

### Results and Conclusions

#### Dissections

Adult physiology can be divided into four clear phases (Figure 1). *Early-1 phase*, which starts when adults appear, is dominated by adult feeding, and the adult sex ratio is close to 50:50 on trunks of trees.



**Figure 1.** Adult phenology of spotted lanternfly.

In *Early-2 phase*, adults continue to feed intensively, but the sex ratio on tree trunks shifts to mostly female. The first observation of mating marks the beginning of the *Mid phase*, which is followed by mass flights and female abdomens swelling markedly with ovaries developing after mating. The first observation of newly deposited egg masses marks the beginning of *Late phase* which is dominated by oviposition.

Measurements of the yellow membrane between the abdominal tergites revealed that male abdomens shrink and female abdomens swell with the onset of mating, as the males transfer a large nuptial gift to females. Mated females had sperm in their spermatheca and their bursa copulatrix contained the nuptial gift.

Ovaries did not start developing until after mating. Mating was first observed in mid-September; 60% of females had mated by the end of September, and 100% by mid-October. The progression of egg loads from large (>30) to medium (15-30) to small (<15) suggests that the entire egg load may not be deposited in a single oviposition event (Figure 2).

*Distribution among host plants*

The combined trees in the five transects were dominated by 22 black walnut and 21 *A. altissima* trees, but also six black locust, five black cherry, three red oak, two white ash, one white pine, and one mulberry tree.

First instars were the most broadly distributed among the different species of host plant, but were inordinately trapped on black cherry (Figure 3). This may be a reflection of where eggs had been deposited the previous season.

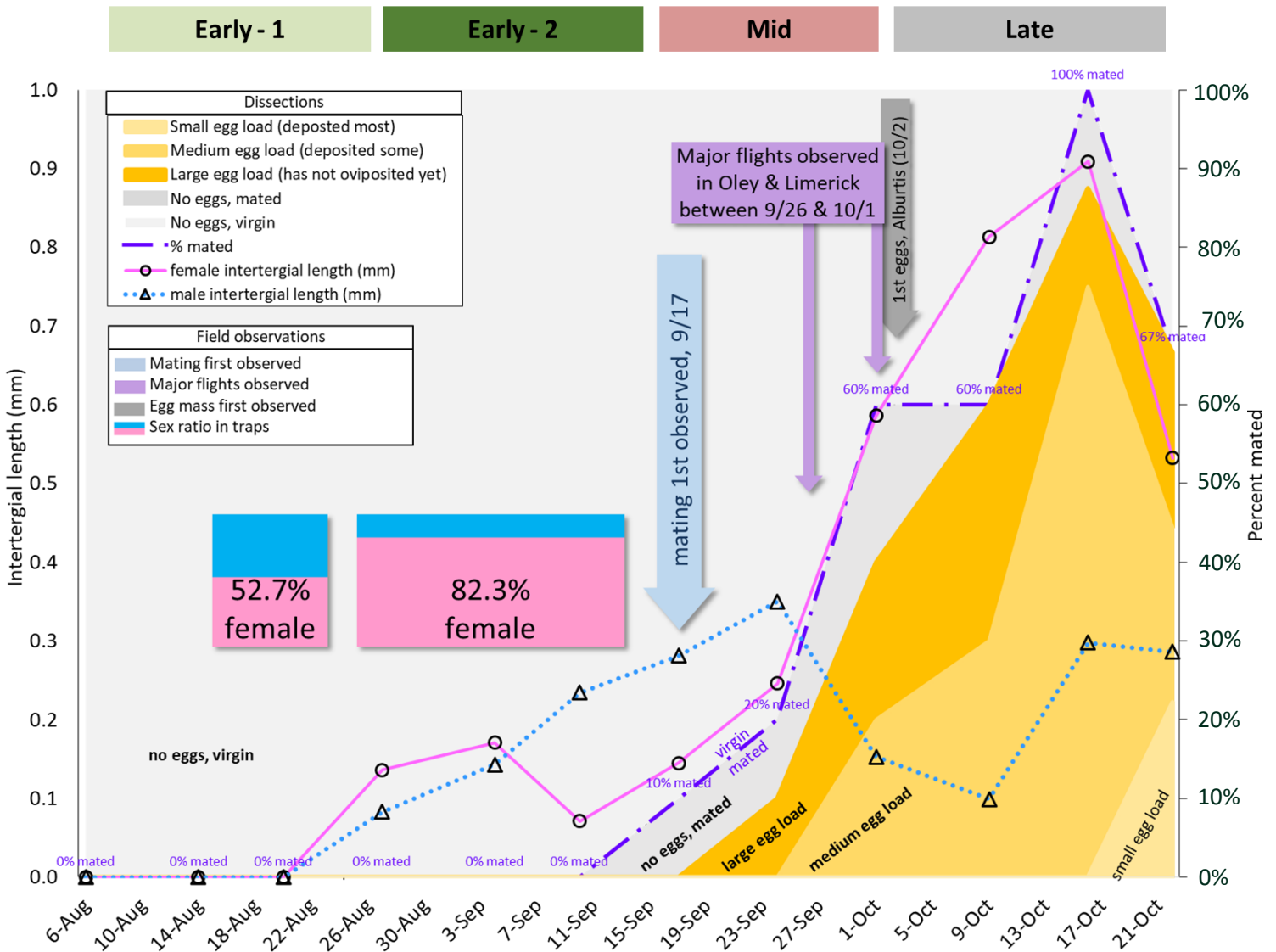
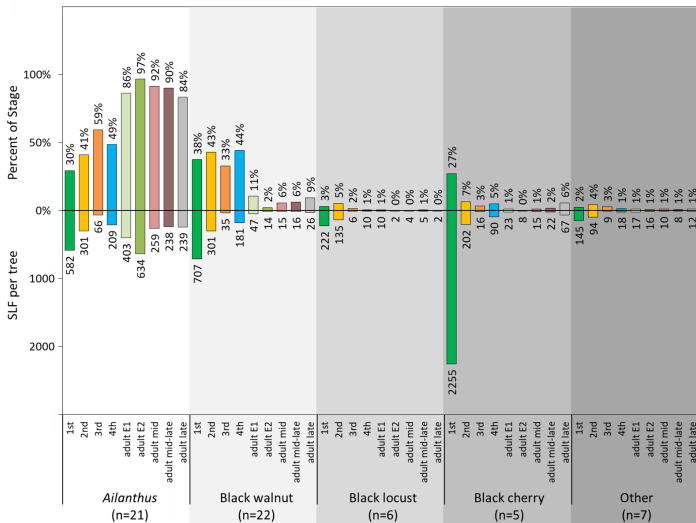


Figure 2. Dissections and measurements alongside field observations of adults over 12 weeks.



**Figure 3.** The percent of each SLF stage that was captured on different tree species in the forest (upward bars), and the average number of SLF captured per tree for each species and instar (downward bars).

The most preferred tree in the forest was *A. altissima*, but black walnut was the second most preferred tree. As SLF developed from stage to stage, their population was increasingly found on *A. altissima*, peaking at the adult Early-2 phase at which time 97% of the entire SLF population in the forest was captured on *A. altissima*. This corresponded to the period of time when the population on tree trunks was predominantly female during the two weeks prior to mating. This suggests that adult SLF may locate *A. altissima* during that two-week period for the purpose of finding mates. It is unclear where males are going during the Early-2 phase.

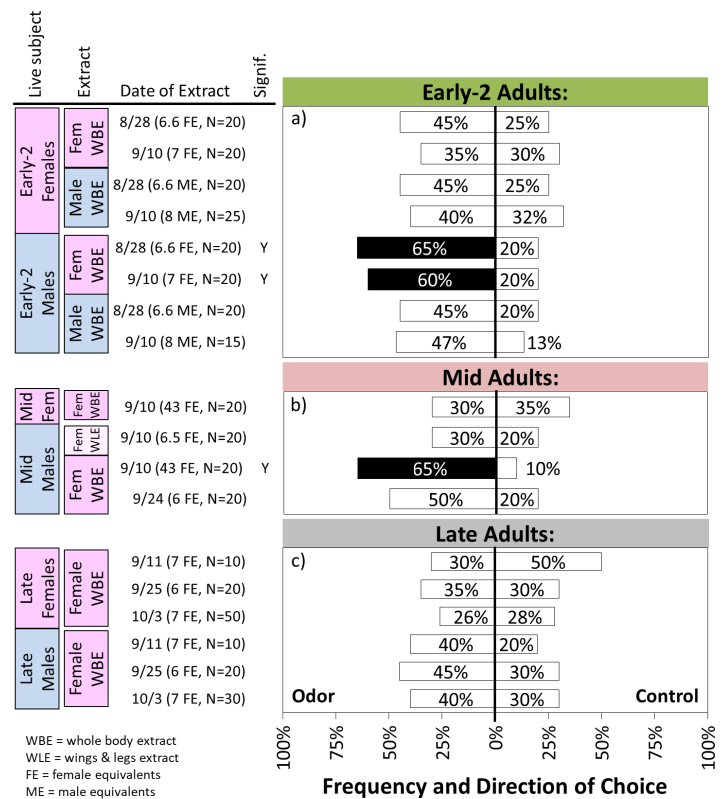
Our observations were limited to the bottom 2 m of trees, so perhaps males head to the canopy at that time. After mating, the percent of adults found on *A. altissima* started to decrease. Females may then leave depleted *A. altissima* trees in search of more vigorous hosts for oviposition.

**Chemical ecology of adults**

Male and female body washes from Early-2 adults were tested in laboratory Y-plate bioassays for attraction by field caught Early-2 males and females. Females were not significantly attracted to the odors from Early-2 males or females. However, Early-2 males were significantly attracted to the odors from Early-2 females, but not Early-2 males (Figure 4a). Mid males were significantly attracted to odors from Early-2 females, but not Mid females, and Mid females were not attracted to Early-2 female odors (Figure 4b).

Late males and females were not attracted to odors from Early-2, Mid, or Late males or females (Figure 4c). Since Early-2 and Mid males were attracted to odors from only Early-2 females which were not mated yet, but not to odors from the stages when females had already mated, there may be a sex pheromone at play. Since no females were attracted to odors from any males or females, we would expect a sex pheromone would be produced by the females.

GC-EAD revealed that male antennae responded to several volatiles from Early-2 females, however, the antennally active compounds were in such small amounts that they have not yet been identified chemically. Future work will focus on collecting very large amounts of female volatiles for the purpose of identifying antennally active compounds.



**Figure 4.** Frequency and direction of choice of male and female SLF of different ages responding to the volatile extracts taken from male or female SLF at different ages in Y-plate walking bioassays. Black bars are significantly different from the white bars in the opposite direction (Chi-square test,  $\alpha=0.05$ ). Early-2 and Mid males, only, were significantly attracted to whole body extracts from Early-2 females, only.

## Spotted lanternfly host preference: Black walnut versus tree-of-heaven

Miriam Cooperband<sup>1</sup>, Miranda Fetchen<sup>2</sup>, and Allison Cornell<sup>2</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

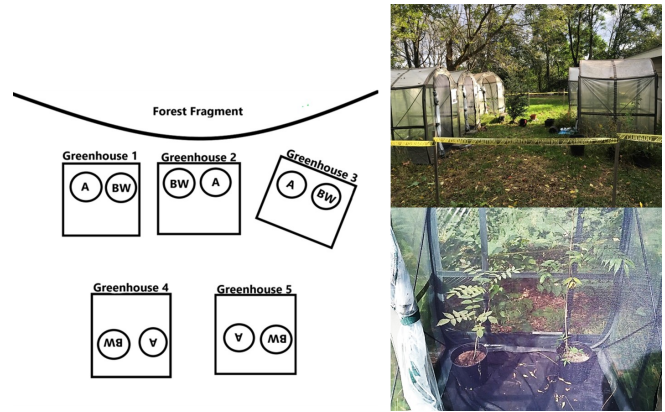
<sup>2</sup>Cedar Crest College, Department of Biology, Allentown, PA

### Introduction

The spotted lanternfly, SLF, *Lycorma delicatula*, is a new invasive pest originating from China that was discovered in Pennsylvania in 2014. SLF have been observed to feed on a broad range of over 70 host plants, but we have found that some host plants are more suitable for feeding, survival, and development than others. Their preferred host, tree-of-heaven, *Ailanthus altissima*, is also invasive from China. To reduce and slow the spread of the expanding SLF population most *A. altissima* are being removed, while the remaining are being treated with insecticide. Our studies have also found that black walnut is a suitable host for survival and development of SLF, even in the absence of *A. altissima*. The goal of the present study is to determine whether SLF will feed on *A. altissima* trap trees when black walnut is available.

### Methods

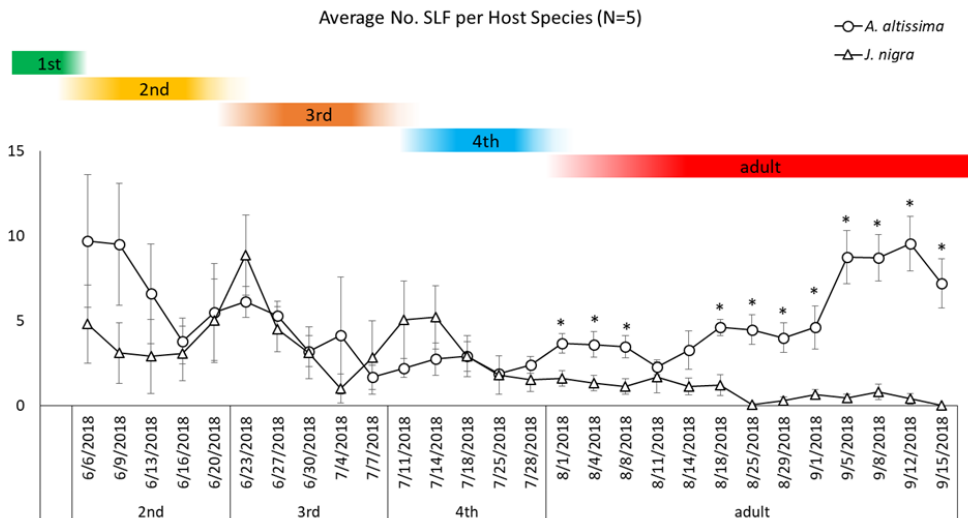
Five field green houses were set up in Allentown, Pennsylvania. Black walnut and *A. altissima* saplings of similar size were dug up from the wild and transplanted into three gallon plant pots and allowed to establish. Each greenhouse received a pair of potted trees containing one *A. altissima* and one black walnut (Figure 1). On May 31, 2018, 30 first instar SLF were field caught and placed into each greenhouse. The number of SLF on each tree was recorded three times per day (morning, afternoon, and early evening), three days per week, for 15 weeks, until September 15, 2018.



**Figure 1.** Diagram of the experimental setup showing the arrangement of greenhouses (left, top), and potted plants within a greenhouse (bottom).

### Conclusions

No difference was found in SLF preference between *A. altissima* and black walnut during early life stages. However, starting on August 1, 2018 with the first appearance of adults, there was a significant preference for *A. altissima* over black walnut. The preference became more pronounced in the first two weeks of September, coinciding with the Early-2 adult phase which precedes mating. Although black walnut is a suitable host for developing SLF, *A. altissima* is strongly preferred over black walnut by adult SLF. These results bolster similar findings in other studies which suggest that *A. altissima* may play an important role in SLF finding their mates.



**Figure 2.** The average number of SLF present per tree over time. Asterisks indicate a significant difference in SLF between tree species.



## Dispersal and host preference of marked and released spotted lanternfly

Miriam Cooperband<sup>1</sup>, Kelley Murman<sup>1,2</sup>, Stefani Cannon<sup>1,2</sup>, Leslie Abreu<sup>1,2</sup>, and Matthew Wallace<sup>2</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

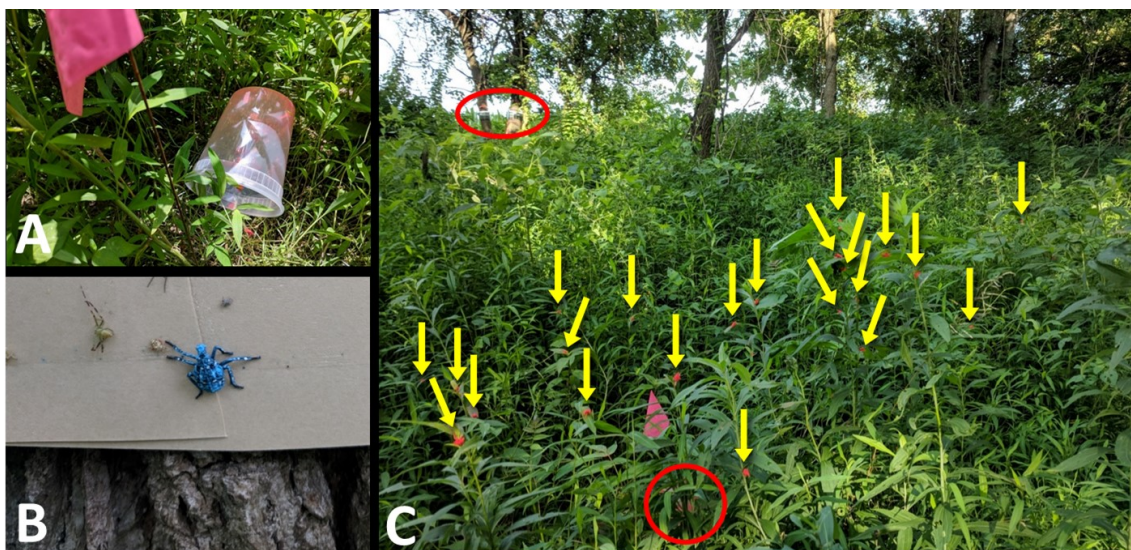
<sup>2</sup>East Stroudsburg University, Department of Biological Sciences, East Stroudsburg, PA

### Introduction

The spotted lanternfly, SLF, *Lycorma delicatula*, is a newly invasive pest native to China that was discovered in Pennsylvania in 2014. SLF have been observed to feed on a broad range of over 70 host plants, but we have found some host plants are more suitable for feeding, survival, and development than others. Their preferred host, tree-of-heaven, *Ailanthus altissima*, is also invasive from China. Removal of most *A. altissima*, and establishing the remaining *A. altissima* as trap trees, is a technique being broadly used to reduce and slow the spread of the expanding SLF population. The optimal spacing of trap trees would depend on the natural tendencies of SLF to move certain distances. Last year we conducted a mark-release-recapture study to see how far SLF move when already on their preferred host. We found that if SLF were on a healthy tree-of-heaven, they tended to stay on it. This year a similar study was conducted to evaluate the questions: 1) how far will SLF move when starting on the forest floor, 2) do they direct their movement towards trap trees, and 3) if so, from how far away?

### Methods

A mark-release-recapture experiment was conducted along five transects on forested and rural residential properties in Pennsylvania. Transects started at a cluster of *Ailanthus* trap trees and extended approximately 80 m. Transects were drawn in a direction that did not intersect trees in the first 10 m away from the trap trees. All trees (>15 cm dbh) surrounding the transect beyond that point were banded with Webcote (1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> instars) and BugBarrier (4<sup>th</sup> instars and adults), including the trap trees. At the beginning of each life stage, SLF were captured, marked with pink, green, or blue fluorescent powder dye, and released on the ground 10, 25, and 50 m, respectively, from the trap trees along the transect (Figure 1). The number of SLF released at the three points were  $2\pi r \times 1.2$ , where  $r$  is the release distance from the trap tree. Therefore, 75, 188, and 377 SLF were released, respectively, at the three points on each transect. Twenty-four hours after each release the positions of marked SLF (2<sup>nd</sup> instar – adults) were observed, and the direction and distance traveled was recorded as well as what host plant species they were on. Bands were also checked after 24 hours and changed if any marked SLF were captured. Bands were monitored biweekly thereafter.



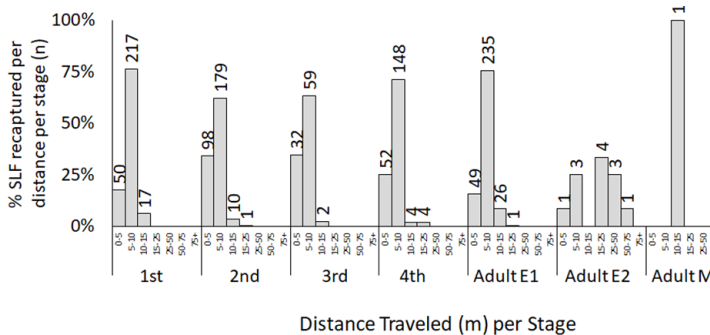
**Figure 1.** Photographs from a transect in a forest. A) Releasing pink marked SLF. B) A blue marked SLF on a sticky band. C) Locations of pink marked SLF after 24 hours (arrows), and their release point (circle) 10 m away from trap trees (oval).

**Results**

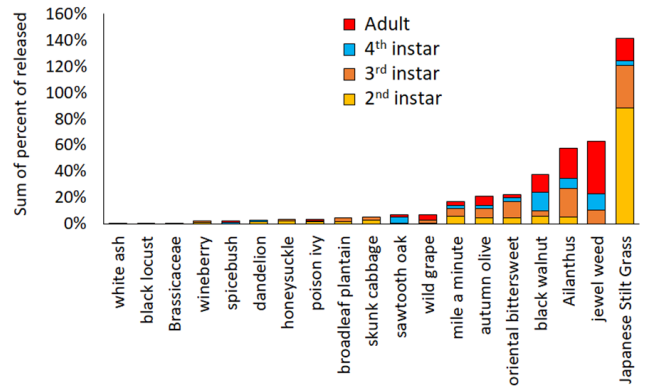
Recaptures on bands two weeks after releases revealed that 94% of all marked SLF were caught within 10 m from their release point, with 24% captured 0-5 m away (five possible trees), 70% captured 5-10 m away (37 possible trees), and 6% captured >10m away (137 possible trees). When this was examined by stage, the same pattern was apparent for all stages, first instars through Early-1 adults (Figure 2). Marked Early-2 adults were at large for four weeks and were captured at greater distances. Only one adult was recaptured (more than six weeks after being released), and she was released at the 10 m distance and recaptured on a trap tree. There were roughly seven times more recaptures on rural residential properties than in forest transects.

In addition to the bands along each transect, Pennsylvania Department of Agriculture provided us with all of their survey bands within a five mile radius from each transect targeting adults from September 6 to November 5, which encompassed the peak period of mass flight. This amounted to 345 bands, none of which captured any marked SLF.

A snap shot of where SLF were located after 24 hours revealed most 2<sup>nd</sup> and 3<sup>rd</sup> instar nymphs were still observed through the tall Japanese stilt grass in the forested plots (Figure 1b). However, excluding that plant revealed significantly more SLF were found on some host plants than others within the same distance (Figure 3). Second instar SLF 1-2 m from their release appeared to prefer mile-a-minute weed and autumn olive, two invasive weeds from Asia. Similarly, 4<sup>th</sup> instar SLF also appeared to prefer invasive weeds from Asia, such as sawtooth oak, oriental bittersweet, autumn olive, mile-a-minute weed, and *Ailanthus*.



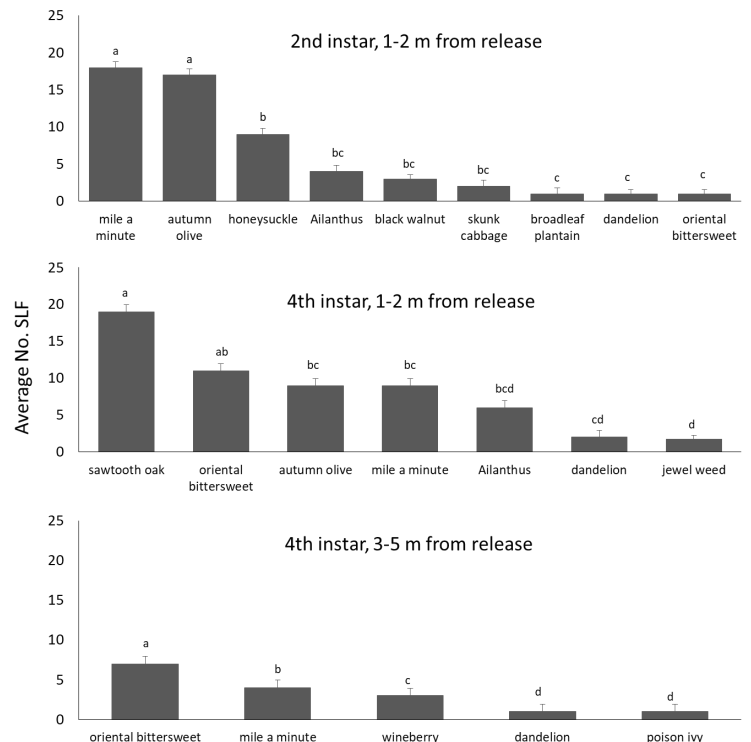
**Figure 2.** The percent (and number) of marked-recaptured SLF, and stages, that travelled different distances.



**Figure 3.** Stacked percentages of which host plants each instar of marked SLF were found on 24 hours after their release in the field.

**Conclusions**

This mark-release-recapture study, similar to the previous study, revealed that before developing wings the majority of SLF are unlikely to travel very far in a 2-week period, with 94% being recaptured less than 10 m from their release point. There was no obvious preference for one species of banded tree over another, they appeared to head for the closest tree. After the first two weeks, adults were found to travel beyond that distance in greater proportions. The SLF that travelled the farthest were Early-2 adults that were captured 54.1 m away from their release point. After 24 hours SLF appeared to show host preferences within the understory, revealing a possible affinity for other invasive weeds from Asia.



**Figure 4.** Distribution of marked-released 2<sup>nd</sup> and 4<sup>th</sup> instar SLF on host plants 24 hours after their release.

## Tree effects: Understanding what makes “hot” trees hot to spotted lanternfly

Miriam Cooperband<sup>1</sup>, Linnea Meier<sup>1,2</sup>, Jacob Wickham<sup>3</sup>, Kelly Murman<sup>1,4</sup>, Stefani Cannon<sup>1,4</sup>, Leslie Abreu<sup>1,2</sup>,

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>University of Florida, Tropical Research and Education Center, Gainesville, FL

<sup>3</sup>Chinese Academy of Sciences, Institute of Chemistry, Beijing, China

<sup>4</sup>East Stroudsburg University, Department of Biological Sciences, East Stroudsburg, PA

<sup>5</sup>Pennsylvania State University, Montgomery County Extension Office, University Park, PA

### Introduction

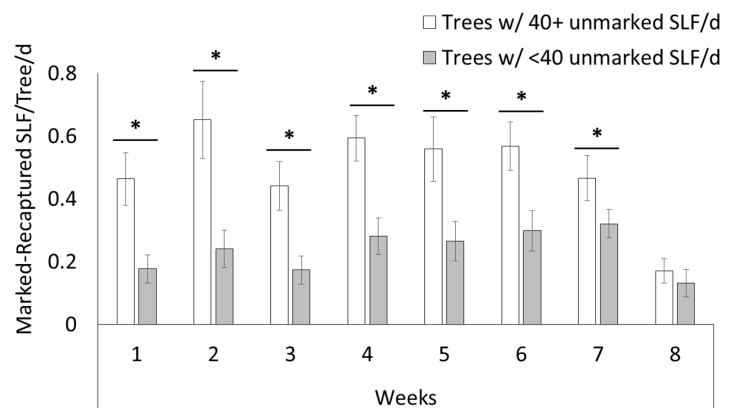
The spotted lanternfly, SLF, *Lycorma delicatula*, is a newly invasive pest native to China that was discovered in Pennsylvania in 2014. They have been observed to feed on a broad range of over 70 host plants, but their preferred host is tree-of-heaven, *Ailanthus altissima*, also invasive from China. As of yet, no pheromones have been identified for this insect, although they do appear to aggregate. These aggregations are observed on trees which appear to be heavily attacked, while virtually ignoring adjacent trees of the same species that are similar in size and vigor (Figure 1). We call this phenomenon “tree effects” for lack of a better explanation, and it has left researchers perplexed as to why SLF appear to strongly prefer some trees over others. Tree effects can interfere when conducting field experiments attempting to test differences between traps, lures, or insecticide treatments. We hypothesize that tree effects may be the result of a feedback loop whereby SLF feeding on a host plant produce a pheromone and/or host plant damage volatiles that attract more SLF, which in turn produce even more of the same volatiles.



**Figure 1.** Two examples of “tree effects”, or non-random distribution, in A) 4<sup>th</sup> instar nymphs and B) egg deposition. A high SLF population is seen on certain trees, whereas similar adjacent trees have very few SLF on them. In the absence of understanding the mechanism for this incongruous distribution of SLF, this phenomenon has been called “tree effects”.

### Methods and Results

A study was conducted in Beijing, China whereby 32 pairs of trees were tested for a period of eight weeks. In each pair, one tree received a methyl salicylate lure, and the other received no lure. Half of the methyl salicylate lures were manufactured by AgBio, the other half were manufactured by Alpha Scents. All lures had similar release rates. Traps were tallied every week, and rotated every two weeks. The results found no significant differences between trees with lures and those without (paired T-test). Further, trees that caught more SLF before the rotations also caught more SLF after the rotations. Therefore, some trees simply had higher SLF populations on them and lures could not override their effects. However, in addition to the trapping of the background population, we also released 20 marked SLF on the ground halfway between each pair of trees, using different colored dye for different pairs. Marked individuals also were not captured in higher numbers on trees with lures. Suspecting tree effects, as a post hoc analysis, the median number of SLF per tree was used to categorize trees as either high (40+ unmarked SLF/d) or low (<40 unmarked SLF/d) population trees. When examining where the marked SLF ended up in this way, they ended up in the high population trees significantly more than in the low population trees for the first seven weeks of the study (1<sup>st</sup>-4<sup>th</sup> instars) (Figure 2).



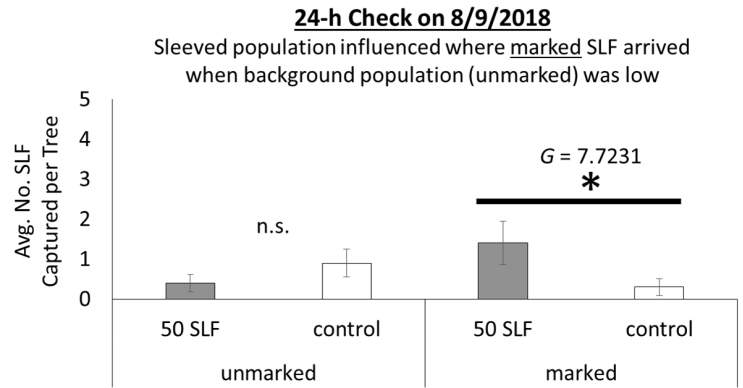
**Figure 2.** Marked 1<sup>st</sup> through 4<sup>th</sup> instar SLF released weekly between pairs of trees oriented towards trees with higher background populations (40+ SLF/d) significantly more than those with lower background populations (<40 SLF/d), overriding effects of high release methyl salicylate lures.



A second study was conducted at a very low density site in Pennsylvania. In this study, pairs of adjacent trees received 3.3 m long sleeves which were either stocked with 50 adult SLF (25 males and 25 females) or were left empty (control). Trees received a BugBarrier sticky band above the sleeves (Figure 3). Because we know from our research in 2017 that the majority of SLF on healthy *Ailanthus* trees tend to stay on the same tree, we marked fifty adult SLF with fluorescent powder and released them on the ground halfway between the two trees to ensure they all started from the same place with an equal opportunity to select either tree in the pair. After 24 hours, the positions of the marked SLF were recorded, and sticky bands were tallied for unmarked SLF as well, indicating the background populations on trees. Background levels were lower than the marked SLF, so would have produced a weaker signal than the SLF in the sleeves. A chi square test was used to compare capture rates on trees with live bait versus control trees. Trees with live bait captured significantly more marked SLF than those with empty sleeves (Figure 4).



**Figure 3.** The experimental arrangement of the “live bait” study, in which marked SLF were released on the ground halfway between pairs of sleeved trees. In each pair one sleeve contained no SLF and the other contained 50 SLF adults. BugBarrier sticky band traps were placed above sleeves.



**Figure 4.** Capture of SLF marked and released between paired, sleeved trees, one containing 50 SLF adults as “bait” and the other an empty control. Unmarked background population was also monitored. Asterisk indicates marked SLF chose trees baited with 50 live SLF significantly more than control trees, whereas the background population (unmarked) did not differ significantly between paired trees (chi square test,  $\alpha=0.05$ ).

**Conclusions**

These studies provide experimental evidence that trees with high numbers of SLF attract significantly more SLF than trees with low numbers of SLF, confirming that “tree effects” are likely produced by the SLF themselves. However, the mechanism for this attraction has not yet been identified, nor has the reason why certain trees are initially selected. Possible mechanisms could be an aggregation pheromone or host damage kairomones. Either of these could produce a feedback loop whereby additional SLF arriving would make the tree that much more attractive. Learning why certain trees are initially selected could improve detection by targeting the trees that are most likely to be attractive for detection activities.



## Dose response and detection of velvet longhorned beetle, *Trichoferus campestris*, populations using attractant baited traps

Joseph A. Francese<sup>1</sup>, Ann M. Ray<sup>2</sup>, Kristopher Watson<sup>3</sup>, Roy Bower<sup>4</sup>, Everett G. Booth<sup>1</sup>, Joey Caputo<sup>3</sup>, Frank Buccello<sup>5</sup>, Emily Franzen<sup>2,6</sup>, Yunfan Zou<sup>7</sup>, Angie Ambourn<sup>8</sup>, Renee Pinski<sup>9</sup>, and Jocelyn G. Millar<sup>7</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>Xavier University, Department of Biology, Cincinnati, OH

<sup>3</sup>Utah Department of Agriculture and Food, Salt Lake City, UT

<sup>4</sup>Xavier University, Department of Mathematics, Cincinnati, OH

<sup>5</sup>New York State Department of Agriculture and Markets, Jamaica, NY

<sup>6</sup>Otis Laboratory Bethel Field Station, USDA APHIS PPQ S&T CPHST, Bethel, OH

<sup>7</sup>University of California at Riverside, Department of Entomology, Riverside, CA

<sup>8</sup>Minnesota Department of Agriculture, St. Paul, MN

<sup>9</sup>Wisconsin Department of Agriculture, Trade and Consumer Protection, Madison, WI

### Introduction

The velvet longhorned beetle, VLB, *Trichoferus campestris*, (Figure 1) is native to East Asia where it feeds on a wide range of tree species, including orchard and timber trees. Larvae of VLB can be transported in wood packing material, and individuals are frequently intercepted at U.S. ports of entry. Populations of VLB have established outside of the native range of the species, including near Salt Lake City, Utah. Adults are nondescript and nocturnal, additionally monitoring and control efforts have been hindered by a lack of attractant lures.



**Figure 1.** Velvet longhorned beetle, VLB, *Trichoferus campestris* (Photo credits Sindhu Krishnankutty).

We recently isolated and identified a novel variant of the conserved 2,3-alkanediol/hydroxyketone chemical structure from headspace volatiles of males but not females. In earlier field bioassays, this compound, which we call trichoferone, attracted significantly more adult beetles than did commercially available high-release ethanol lures or solvent control [1].

### Response of VLB to varying release rates of trichoferone

This year, we aimed to determine the optimal release rate for commercially manufactured lures of trichoferone. Determining the optimal release rate would allow for a lure to be delivered to CAPS for future surveys. Field bioassays were undertaken by placing traps in an active peach, cherry, and apple commercial orchard in Pleasant Grove, UT. Black intercept panel traps were baited with one of three release rates of trichoferone (1 mg/d, 3 mg/d, and 9 mg/d), or a blank control. Traps were deployed ~10 m apart in approximately linear transects. Traps were checked and treatments rotated (to control for positional effects) every week. Lures with a 9 mg/d release rate caught more beetles than any other treatment, while 3 mg/d lures caught more beetles than 1 mg or blank traps (Table 1). Because of the amount of material needed and the potential cost per lure, 3 mg/d will be a sufficient lure release for attraction and survey needs.

### Update of detections of VLB outside UT using trichoferone

Traps baited with trichoferone were also tested by state co-operators, surveyors and Otis staff in six other states.

- New York: 74 trichoferone-baited traps were placed at nine locations in Westchester County. Seven of these locations had been used in previous years. Samples are still being identified from these traps, but as of this writing, 167 VLB were caught in 63 traps, placed at all nine locations.

**Table 1.** Mean VLB trap catch per week (and standard deviation) in a comparison of three release rates of trichoterone tested in an active commercial peach, cherry, and apple orchard in Pleasant Grove, Utah. A Kruskal-Wallis rank sum test was utilized to determine if any differences occurred between lure types. A Dunn's pairwise comparison was performed to compare VLB caught across each lure type.

Lure (N)	Blank (150)	1mg/d (148)	3mg/d (148)	9mg/d (148)
Mean (SD)	1.53 (2.33)	1.50 (2.31)	2.03 (5.94)	6.84 (7.19)

- Ohio: 10 trichoterone-baited traps (2 each at 1 mg/d, 3 mg/d, 9 mg/d, a blank control and a second set of 3 mg/d from a second manufacturer) were placed in East Fork State Park in Bethel, where beetles had been previously caught. A total of eight VLB were caught, with a majority caught in 3 mg/d traps.
- Minnesota: Five trichoterone baited intercept traps were placed at sites in the Minneapolis area where VLB had previously been caught. After one week in the field, 13 VLB were caught, and the surveyors rotated the traps to new locations. More lures were provided to them, and in total 92 VLB were captured at 19 locations.
- Wisconsin: 10 trichoterone-baited traps were set up. A total of 74 beetles were collected in eight traps, with some being as many as 10 miles apart. This was an increase from 2017, when only two beetles were caught at two locations. It is worth noting that traps were left in the field for six to eight weeks (2-4 weeks beyond the life expectancy of the lures) and multiple traps still collected beetles during the later weeks.

- Massachusetts and New Hampshire: A total of 14 traps were placed (13 and one, respectively). There had been no previous detections in either state, and no beetles were caught.

### Conclusions

A 3 mg/d trichoterone, fluon-coated intercept panel trap has been recommended as the CAPS survey method for VLB. Lures will be made available in 2019 for use in state surveys. Our plan is to test this lure against our trichoterone standard in Utah, New York, and Ohio. VLB have been shown to be responsive to light traps in the past, so we will also test a new, promising light trap design with VLB.

### Reference

- 1) Ray, AM, Francese JF, Zou Y, Watson K, Crook DJ, Millar JG. Isolation and identification of a male-produced aggregation-sex pheromone for the velvet longhorned beetle, *Trichoterus campestris*. Scientific Reports 2019 March 14; 9: 4459, doi: [10.1038/s41598-019-41047-x](https://doi.org/10.1038/s41598-019-41047-x)

## Developing traps for spotted lanternfly

Joseph A. Francese<sup>1</sup>, Miriam F. Cooperband<sup>1</sup>, Kelly M. Murman<sup>1,2</sup>, Stefani L. Cannon<sup>1,2</sup>,  
Leslie Abreu<sup>1,2</sup>, Everett G. Booth<sup>1</sup>, Matthew S. Wallace<sup>2</sup>, and Sarah M. Devine<sup>1,3</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>East Stroudsburg University, Department of Biology, East Stroudsburg, PA

### Introduction

Spotted lanternfly, SLF, *Lycorma delicatula*, is a phloem-feeding fulgorid generalist from China that was recently discovered in the United States. It is a serious pest of grapes and other cultivated tree crops. SLF has been documented to cause branch dieback on walnut and kill cultivated grape and hops plants. Trapping technology for SLF has relied on the wrapping of sticky bands around tree trunks of the primary host, *Ailanthus altissima*. Sticky bands are messy and need to be replaced often, as they become covered in both target and non-target insects and debris. While relatively effective at capturing first and second instar nymphs, they have shown limitations in their ability to capture later nymphal stages and adults, which have shown a tendency to avoid walking on the sticky surface. Another limitation of using sticky bands is that manufacturers can also change their glue formulations without notice, potentially causing possible changes in insect capture rates. In order to overcome the limitations of sticky band traps and improve trap efficacy for SLF we need to increase trap technology by designing novel traps and redesigning commercially available traps. In addition, we hope to move away from single-use sticky based traps, if possible, and design traps that capture more late stage SLF and less non-target by-catch.

### Webcote vs. Bug Barrier: Comparison of sticky bands

Prior to 2018, sticky bands, including Webcote (Figure 1) were used to monitor for SLF populations. With these trap types, later instar nymphs and adults were not as easily captured as early instar nymphs.

In 2018, a new trapping system, BugBarrier (Figure 2) was tested. This trap is designed to prevent insects from walking over the glue-coated material while climbing the tree. Insects will either be directed across fiber batting, onto the glue-coated material, or, because the glue faces inward, caught on their dorsum or wing. Following testing, more SLF nymphs and adults were captured, and in low density sites more detections occurred, on BugBarrier bands than on Webcote bands (paired *t*-test;  $P = 0.01$ ).

### Bug Barrier vs. Modified Circle Trunk Trap

Circle trunk traps, a non-sticky trap to exploit the walking behavior of SLF, were modified from pecan weevil traps. Pecan weevil trap collection cups were replaced with a 1.9 L screw-top plastic jar (Figure 3). A pesticide strip was placed inside the collection container to knock down and kill the captured SLF. The opening of the trap was also expanded from 0.6 cm to 1.5 cm.



**Figure 1.** WebCote sticky tree band wrapped around an *Ailanthus* host. The trap shown captured many early instar (1st and 2nd) nymphs.



**Figure 2.** BugBarrier sticky tree band on an *Ailanthus* host. The glue coating faces inward and the batting funnels climbing insects toward it.



**Figure 3.** A circle trunk (modified pecan weevil trap) placed on an *Ailanthus* host. The collection jar size has been increased and the entrance has been widened to accommodate large SLF.



These traps were then compared with BugBarrier bands, with a tree rotation halfway through the trapping period, so that all trees tested both trap types. More late instar nymphs and early adults (paired *t*-test;  $P = 0.02$ ) as well as mid adults ( $P = 0.0003$ ), and late adults ( $P = 0.02$ ) were caught in circle trunk traps than on BugBarrier bands. At later periods in the year, the collection jars on some traps filled up over the course of a two week period (Figure 4).



**Figure 4.** A circle trunk collection jar removed from a trap following two weeks in the field during adult flight.

### Tall Traps

As SLF fly from one woodlot to another, they may use tall structures, such as trees or telephone poles as landing points along their journey. We tested modified brown prism traps that consisted of 2 panels (1.22 m x 0.35 m x 3 sides each) stacked on top of each other (for a total height of 2.44 m). One trap was placed along the edge of an infested wood lot and compared with another trap placed 15 m outside the woodlot in an adjacent field (Figure 5).

Traps were baited with methyl salicylate. Each of the six panel faces were marked with an individual number in tape. Photographs were taken at each check interval (every two weeks), and SLF were counted from the photographs so that data collectors would not have to remove each adult from the trap in the field. Top panels collected fewer SLF than bottom panels, suggesting that SLF that fell to the ground climbed up the nearest tall object. Tall traps along the forest edge caught more SLF than those 25 m away in clearings, which is consistent with capture patterns of other forest dwelling insects.

### Using Flight Intercept Traps to Capture Adults

Black intercept panel traps coated in fluon were compared with BugBarrier to determine if black intercept traps can be used for capturing flying adults. Intercept panel traps were placed approximately 5 to 8 m above the ground in the lower/middle canopy of an *Ailanthus* tree. More SLF were caught on BugBarrier bands than on intercept panel traps (paired *t*-test;  $P < 0.05$  over all trapping periods).

### Conclusions and Plans for 2019

Circle trunk traps show promise as a detection tool for SLF. In 2019 we will test new collection methods that may allow for a larger collection volume. We will also compare catch between BugBarrier and Circle trunks at all life stages, and in lower population densities than in 2018. Flight intercept traps, while not shown to be more effective than BugBarrier traps, will be fitted with lights to determine if light plays a role in catch, as some species of plant hoppers have been shown to be attracted to light. A small batch of circle trunk traps will be tested by the SLF program in 2019.



**Figure 5.** Two “tall traps” being placed along the edge of an SLF-infested woodlot and 15 m into an adjacent field.



Discovery of an aggregation sex pheromone and biologically active host odor compounds for *Bactrocera minax*Allard Cossé<sup>1</sup> and Hannah Nadel<sup>1</sup><sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA**Introduction**

The Chinese citrus fly, *Bactrocera minax*, is the most destructive citrus grove pest in south-central China, and can cause up to 100% fruit damage in severe situations (Figure 1). With increased global trade of citrus from China to different parts of the world there is a possibility that fresh, infested citrus fruits from China will reach the U.S. market in the near future.

Currently there are insufficient data on effective lures and survey tools for early detection of Chinese citrus fly. Early detection method would be especially important in the event of detection in the U.S. The present study addresses this lack of knowledge by investigating possible pheromone and host odor attractants.

This ongoing work is a collaboration between APHIS-PPQ, NSF Center for Integrated Pest Management at North Carolina State University, and scientists from the Guangdong Institute of Applied Biological Resources, Hunan Academy of Agricultural Sciences, and Yong Shun County Department of Agriculture in China.



**Figure 1.** A) Male and B) female *Bactrocera minax*.

**Insects**

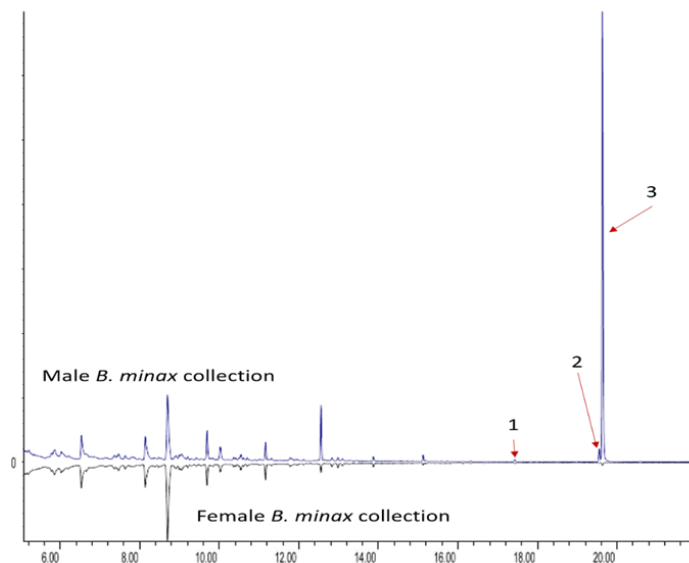
In 2018 Otis Laboratory received approximately 2,000 pupae that were collected by our collaborators in China. These pupae were kept in moist sand and chilled (10°C) for 35 (800 pupae), 45 (800 pupae), and 60 (400 pupae) days. After the chilling period, pupae were placed at 25°C until adult flies emerged (after approximately 27 days). Emerging virgin flies were sexed and placed either individually or as groups on two different diets (wet or dry) consisting of two types of

nitrogen-containing extracts (hydrolyzed yeast and proteose peptone) mixed with sucrose. Flies on dry diet had access to a water wick.

**Volatile collections**

A vacuum system was used to trap volatiles from individual flies and immature sweet orange fruits, *Citrus sinensis*. In the case of *B. minax*, volatile profiles of virgin male and female collections (24h), collected at different times after emergence, were compared by gas chromatography-coupled-mass spectrometry (GC-MS) (Figure 2). The volatile collections from sweet orange fruits were also analyzed by GC-MS. Compounds of interest were identified by micro-chemical reactions and verified with authenticated standards.

The GC-MS comparison showed that virgin male flies produce three predominately male-specific compounds that starts to be detected 11 days after emergence and steadily increases until about 3 weeks, which is when the flies reach sexual maturity and mating commences. Female virgin flies produce at least one of the male-specific compounds but at relatively low amounts. The GC-MS analysis of sweet orange fruit volatiles showed a well-known volatile profile with limonene as the major (97%) odor component.

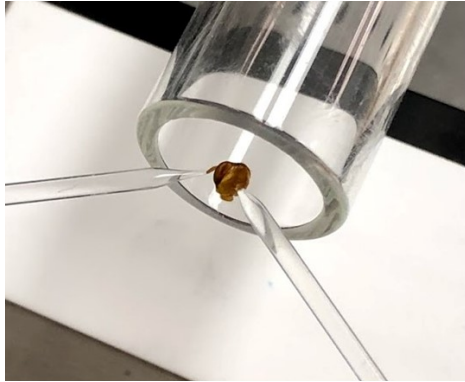


**Figure 2.** GC-MS comparison of virgin male and female *B. minax* volatile collections.

### Electrophysiology

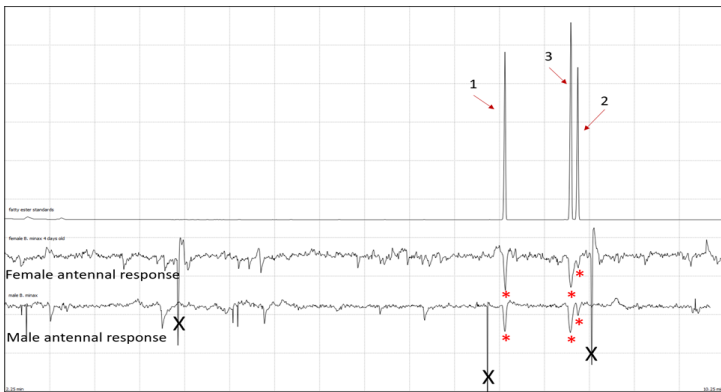
In order to identify specific semiochemicals from volatile collections of flies and immature sweet oranges, a gas chromatographic-coupled-electroantennographic (GC-EAD) methodology was developed, which uses the antennae of male and female flies to detect any biologically active compounds.

For this, the head of the fly was removed and placed on a saline-filled reference electrode, and a second saline-filled recording electrode was brought into contact with the tip of one of the antennae (Figure 3).



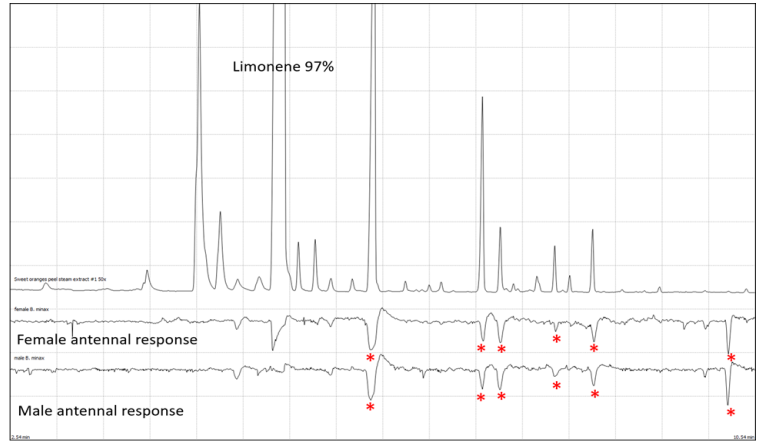
**Figure 3.** GC-EAD antennal preparation for *B. minax*.

This setup was placed in front of the split effluent of the GC, thus recording simultaneously the responses from the antenna and the GC detector output. The GC-EAD analysis showed that male and female antennae consistently responded to three male-produced compounds (Figure 4) and that other detected compounds, previously identified as pheromone components of *Bactrocera* species, did not produce antennal responses.



**Figure 4.** GC-EAD antennal responses of male and female *B. minax* to three synthetic pheromone components (red stars denotes a positive response, X denotes electrical noise).

The GC-EAD analysis of sweet orange fruit odors (Figure 5) showed that male and female antennae respond to at least six different compounds. Even though limonene was the major constituent of the volatiles collected, the antennae responded mainly to the relatively minor components.



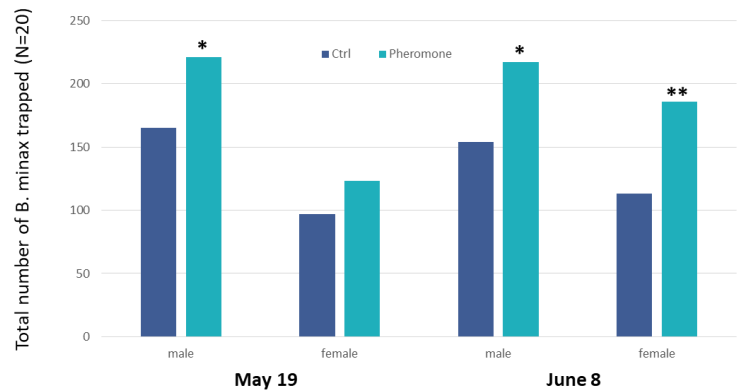
**Figure 5.** GC-EAD antennal responses of male and female *B. minax* to collected sweet orange odor (red stars denotes a positive response).

### Field assay

A preliminary pair-wise field study of baited and un-baited green sticky spheres (Figure 6) showed that a two-component pheromone bait attracted significant numbers of male and female flies in China (Figure 7). Based on the above-mentioned data we can conclude that male *B. minax* produces a male-specific aggregation sex pheromone. Upcoming field tests will further investigate if host odors and pheromones can be used as attractants for the detection of *B. minax* populations.



**Figure 6** Pheromone baited sticky trap.



**Figure 7** Field trapping results (\* denotes  $P < 0.05$ , \*\*  $P < 0.001$ , pair-wise t-test).

**$\beta$ -Ylangene: an attractant for spotted lanternfly from stressed tree-of-heaven bark and foliage**Allard A. Cossé<sup>1</sup>, Nathan Derstine<sup>1,2</sup>, Isaiah Canlas<sup>1,2</sup>, Sam Stella<sup>1,3</sup>, Daniel Carrillo<sup>2</sup>, and Miriam F. Cooperband<sup>1</sup><sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA<sup>2</sup>University of Florida, Tropical Research and Education Center, Gainesville, FL<sup>3</sup>Xavier University, Department of Biology, Cincinnati, OH**Introduction**

The invasive spotted lanternfly, SLF, *Lycorma delicatula*, is a sap-sucking insect native to China, India, and Vietnam. Its primary host is tree-of-heaven, *Ailanthus altissima*, but nymph and adult stages can damage/kill more than 70 tree and plant species, including black walnut, grape, and hops. It was first reported in Berks County, Pennsylvania in 2014, but it is spreading to neighboring states (Delaware, New York, New Jersey, Virginia). There is an urgent need for attractants in survey and detection.

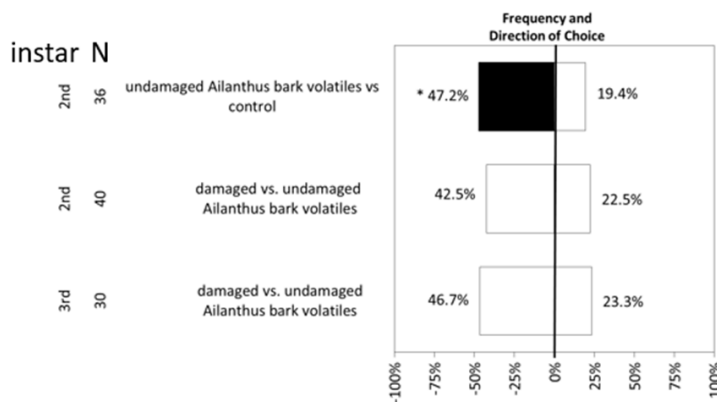
We have identified a kairomone,  $\beta$ -ylangene, in volatile collections from SLF infested *A. altissima* bark and foliage, but also from un-infested, mechanically damaged bark. The attractiveness to  $\beta$ -ylangene to nymphal stages of SLF was demonstrated in a Y-tube olfactometer behavioral bioassay.  $\beta$ -ylangene was detected in volatile bark collections five days after mechanical damage and preliminary data suggests that it might be correlated with tree diameter—with smaller trees and/or with greater relative amount of damage, releasing higher amounts of  $\beta$ -ylangene compared to trees with a bigger diameter or less relative damage.

Identification of  $\beta$ -ylangene was aided by coupled gas chromatographic-mass spectrometric (GC-MS) analysis, GC coupled electroantenna-graphic detection (GC-EAD), purification by high pressure liquid chromatography (HPLC), and GC and MS comparisons with authentic standards.  $\beta$ -ylangene, from volatile collections and from purified natural essential oil, showed antennal activity by GC-EAD using nymphal antennae.

**Methods and Results**

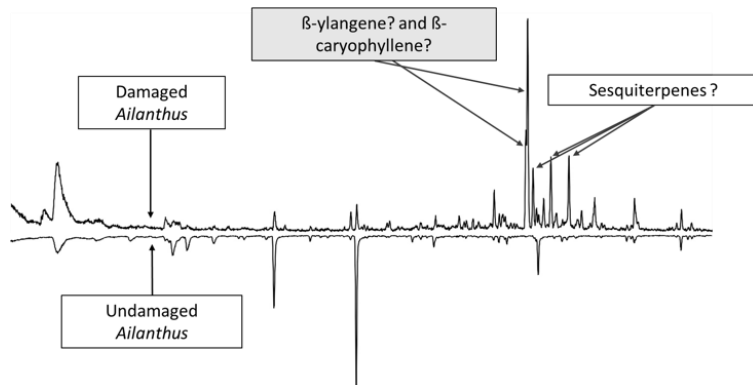
Anecdotal information from the survey crews at the Pennsylvania Department of Agriculture suggested higher numbers of trapped SLF on mechanically damaged *A. altissima* trees. To address this observation we explored several questions. The first question we asked

was if mechanical damage of *A. altissima* trees releases SLF-attractive volatile compounds. We investigated this by using a Y-tube behavioral assay (Figure 1). The results showed that damaged and undamaged bark volatiles did indicate some attractancy to early instar SLF.



**Figure 1.** Behavioral responses (Y-tube olfactometer) of 2<sup>nd</sup> and 3<sup>rd</sup> instar SLF to bark volatiles from undamaged and damaged *A. altissima* trees (\* denotes significant response).

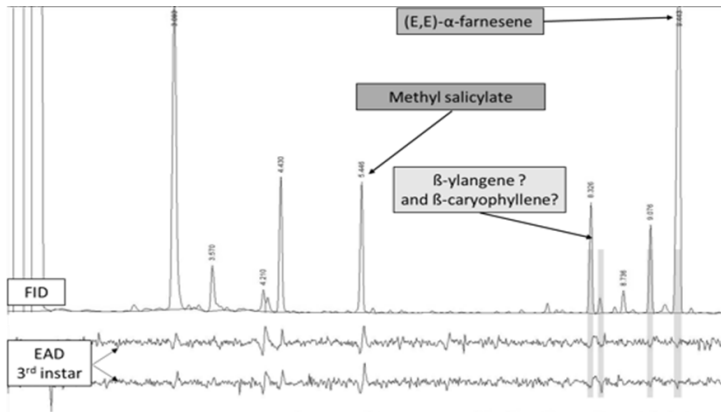
The second question focused on whether there were any specific volatile compounds associated with damaged bark. A comparison by GC-MS of volatiles collected from damaged and undamaged *A. altissima* bark showed that damage increased the release of several compounds called sesquiterpenes (Figure 2).



**Figure 2.** Comparison of GC-MS profiles of bark volatiles from damaged and undamaged *A. altissima*.



A third question addressed whether these sesquiterpenes were also released by SLF feeding and whether the sesquiterpenes were perceived by the SLF antennae. Volatiles were collected from SLF infested *A. altissima* bark and foliage. These volatiles were analyzed by GC-EAD, which uses the antennae of SLF to detect any biologically active compounds. The GC-EAD analysis showed that SLF feeding caused the release of several antennally active compounds, including several sesquiterpenes (Figure 3).

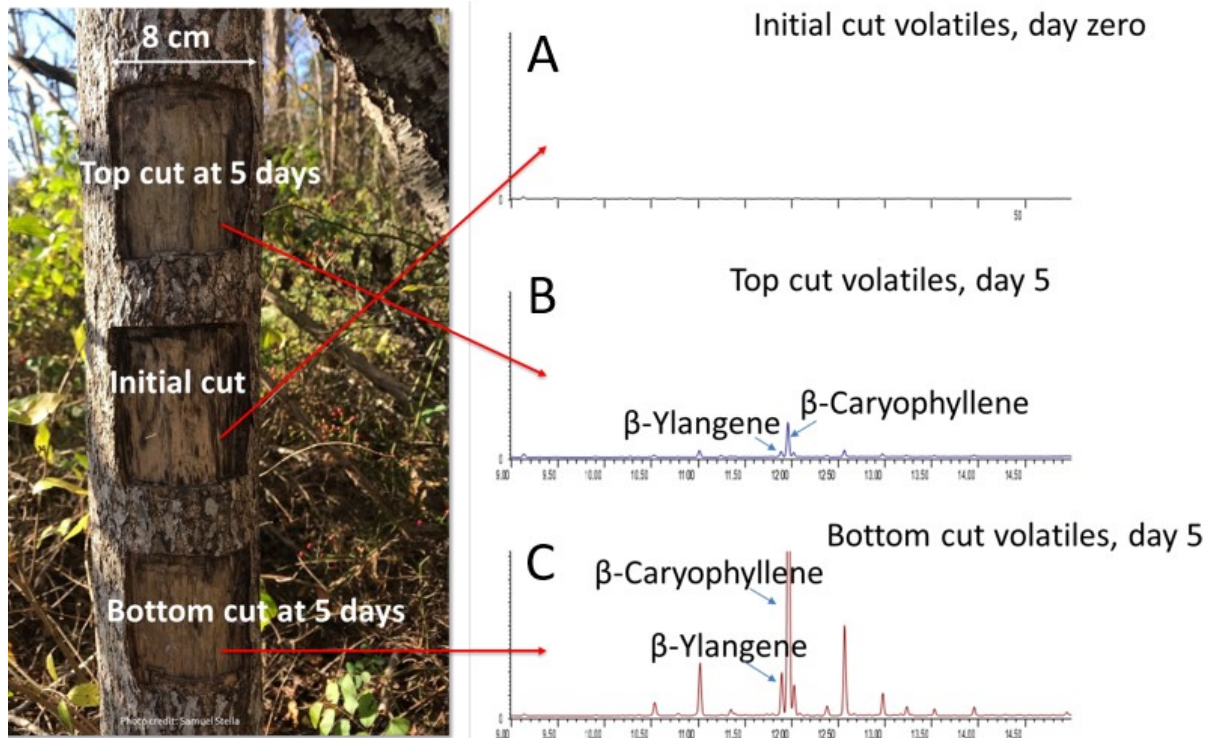


**Figure 3.** Volatile collections from SLF infested *A. altissima* bark and foliage and GC-EAD analysis of volatile collections from SLF infested *A. altissima* foliage (blue bars denotes positive antennal responses to sesquiterpene compounds).

A fourth question concerned the chemical identities of the damage-induced sesquiterpenes and whether these antennally active compounds were also behaviorally active. To address this question several *A. altissima* trees received a bark cut on day zero and volatiles were collected from these bark pieces. This was followed by two more bark cuts (below and above the day zero cut) after five days and again these volatiles were collected and analyzed. The GC-MS analysis showed a relatively strong increase of sesquiterpenes, especially in bark volatiles collected from the bottom cut of the damaged *A. altissima* trees (Figure 4, Table 1).

**Table 1.** Identification and release rates of *A. altissima* bark volatiles from three (T1, T2, T3) different diameter trees (DBH).

Compound	Identification	T1 Top cut ng/day/g dry weight	T1 Bottom cut ng/day/g dry weight	T2 Top cut ng/day/g dry weight	T2 Bottom cut ng/day/g dry weight	T3 Top cut ng/day/g dry weight	T3 Bottom cut ng/day/g dry weight
α-Cubebene	MS + standard	1.4	11.2	0.8	1.0	0.8	0.8
α-Copaene	MS + standard	4.3	31.6	1.9	2.7	2.1	2.0
β-Cubebene	MS	0.6	5.5	0.2	0.5	0.3	0.3
β-Ylangene	MS + standard	2.7	26.3	1.0	1.5	1.1	1.2
β-Caryophyllene	MS + standard	193.3	272.1	7.9	12.6	9.6	9.1
Germacrene-D	MS	1.5	18.5	0.6	0.8	0.8	0.8
α-Humulene	MS + standard	3.8	50.1	1.6	2.4	1.8	1.8
DBH (cm)		38.1		79.6		58.1	
% girdle		20.1		10.0		13.8	



**Figure 4.** GC-MS analysis of *A. altissima* volatiles collected from A) bark cut at day zero, B) top bark cut after 5 days, and C) bottom bark cut after 5 days.



We were able to isolate and purify one of the sesquiterpenes,  $\beta$ -ylangene, from the essential oil of *Cananga odorta* (ylang-ylang oil) using silica gel liquid chromatography followed by HPLC (Figure 5). The purified  $\beta$ -ylangene was tested in the Y-tube behavioral assay and the results showed that 4<sup>th</sup> instar SLF are significantly attracted to this purified material (Figure 6).

**Conclusion**

$\beta$ -ylangene was induced by feeding SLF and mechanically damaged bark, and was attractive to 2<sup>nd</sup>-4<sup>th</sup> instar SLF. Future field work will evaluate the utility of damaged *A. altissima* as a survey tool.

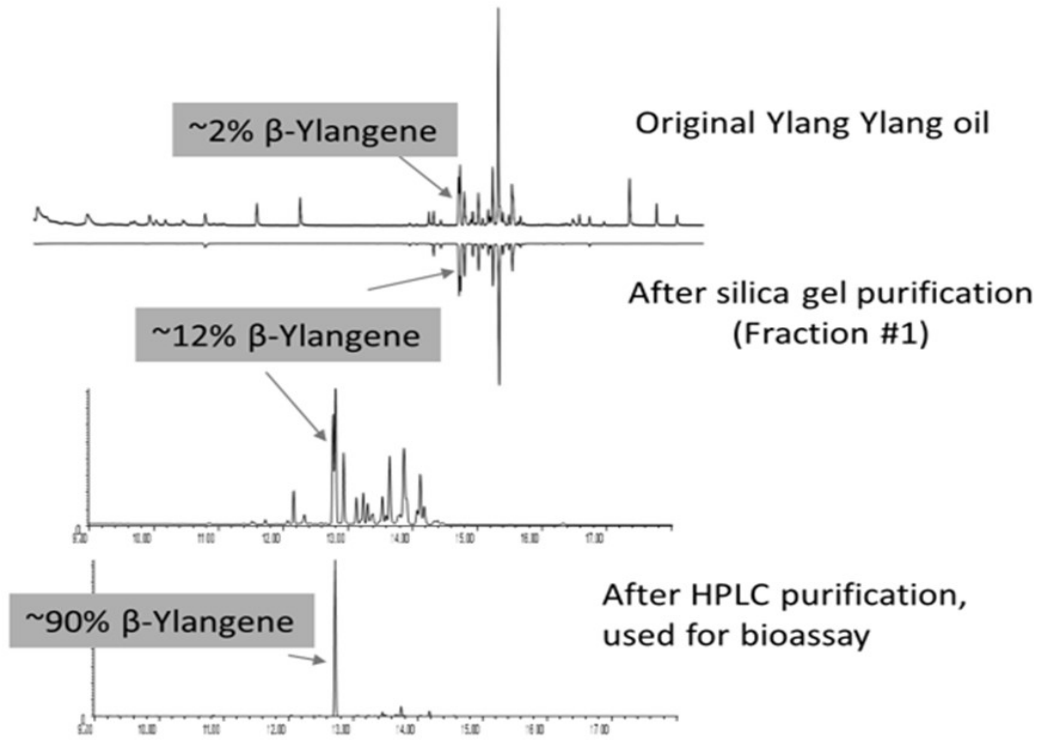


Figure 5. Silica gel & HPLC purification  $\beta$ -ylangene from *Cananga odorta* (ylang ylang oil) essential oil.

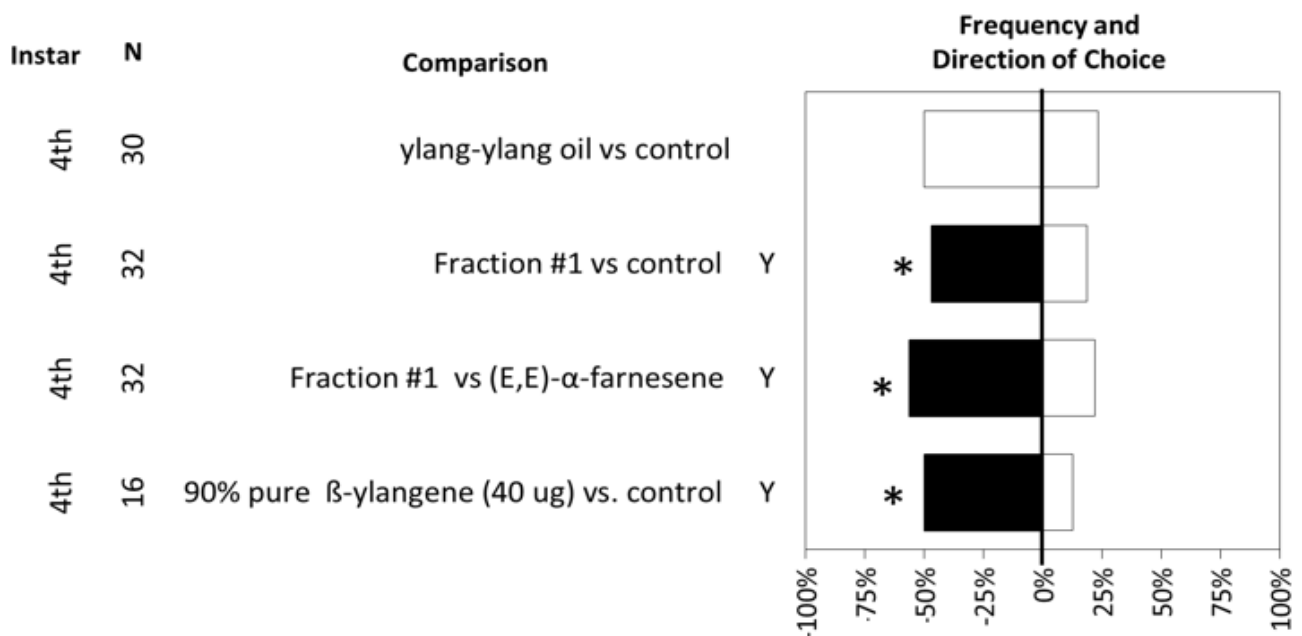


Figure 6. Behavioral responses (Y-tube olfactometer) of 4th instar SLF to ylang ylang oil (1 mg) fraction #1 (1 mg) and purified  $\beta$ -ylangene (0.04 mg) (\* denotes significant response).

## Otis Laboratory CAPS lure support

Allard Cossé<sup>1</sup>, Julia Mackay<sup>1</sup>, and Natalie Leva<sup>1</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

### 2018 Overview

In 2018 the Otis Laboratory formulated more than 100,000 specialized insect lures to all fifty states and two U.S. territories (Figure 1). These lures were prepared in support of the Cooperative Agricultural Pest Survey (CAPS) program. The CAPS program is tasked with providing a survey profile of exotic plant pests in the United States deemed to be of regulatory significance to the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), State Departments of Agriculture, tribal governments, and other cooperators through early detection and surveillance activities. The Otis Laboratory CAPS support involves quality control of insect attractant chemicals, formulation, release analysis, and field efficacy. In addition to the quality control efforts, the Otis Laboratory evaluated the lure efficacy for the European grapevine moth eradication program, European and Asian gypsy moths, and individual research projects of Otis research collaborators by supplying novel experimental formulations (Figure 2).



Figure 2. Collection of volatile materials from insect lure formulations

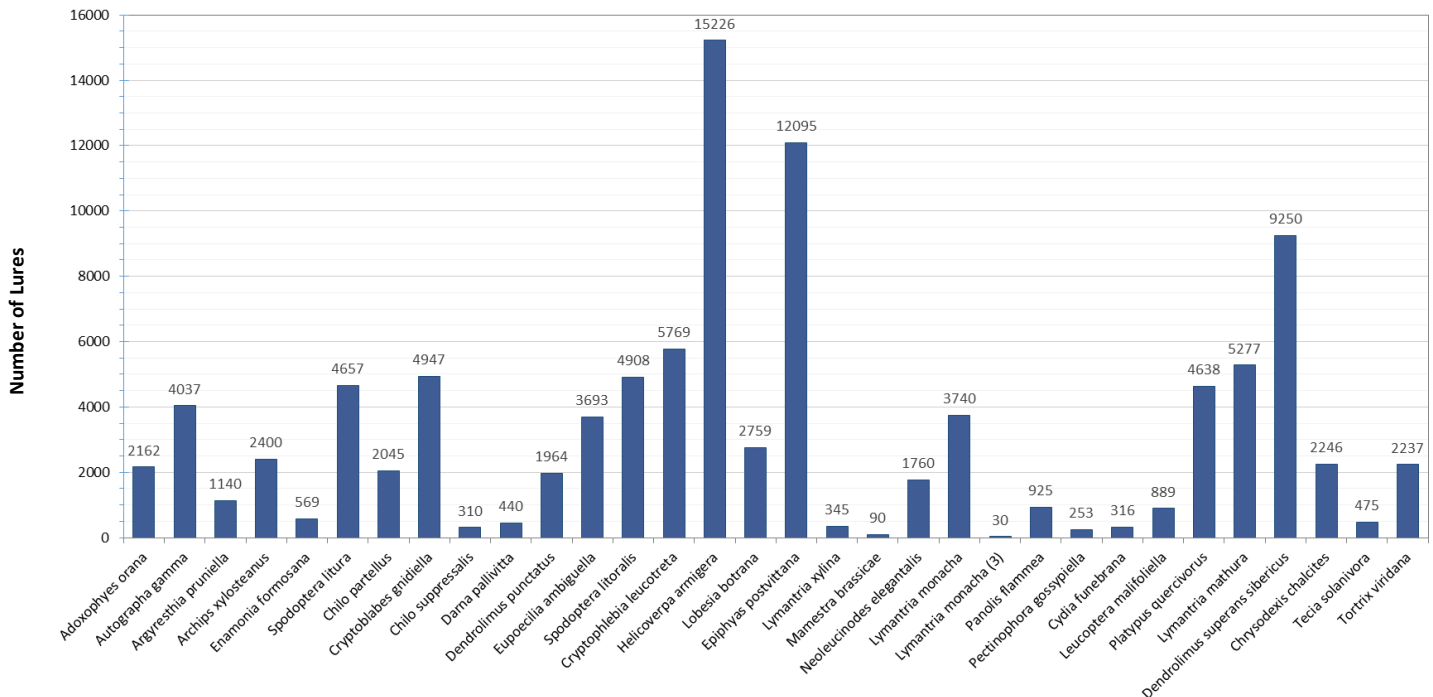


Figure 1. Number of lures formulated for different insect species in support of the 2018 CAPS program.

## 2018 Gypsy Moth Molecular Diagnostics Survey

Kendra Vieira<sup>1</sup>, Nevada Trepanowski<sup>1</sup>, Yunke Wu<sup>1,2</sup>, and Marjorie Palmeri<sup>1,3</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>Cornell University, Department of Ecology and Evolutionary Biology, Ithaca, NY

<sup>3</sup>University of Massachusetts Amherst, Department of Environmental Conservation, Amherst, MA

### Introduction

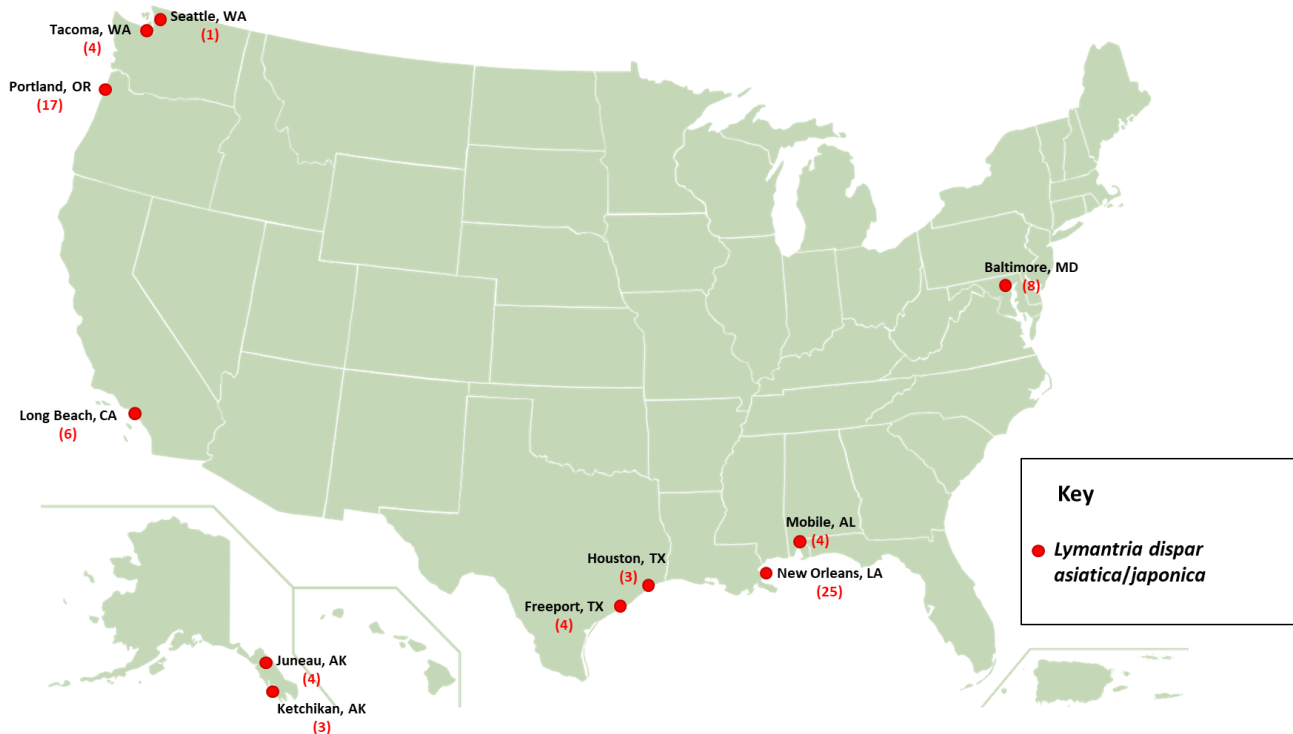
The Gypsy Moth Molecular Diagnostics Survey originated in 1992 and continues to serve as the primary diagnostic tool for detecting possible introductions of Asian gypsy moth, AGM, *Lymantria dispar asiatica/japonica* in the U.S. The survey is composed of two separate monitoring efforts—the port survey and the domestic survey. The port survey targets suspect AGM of varying life stages that have been intercepted at ports of entry on vessels and cargo. The annual domestic survey is conducted in conjunction with the gypsy moth flight season and aims to identify AGM present in the U.S. Additionally, the domestic survey offers valuable insight regarding the spread of the established European gypsy moth, EGM, *Lymantria dispar dispar*, in areas outside of the federal EGM quarantine.

Suspect specimens, either intercepted at ports or trapped domestically, are sent to Otis Laboratory for molecular identification. Though morphologically indistinguishable, AGM is distinguished from EGM using the Standard Diagnostic Assay, which

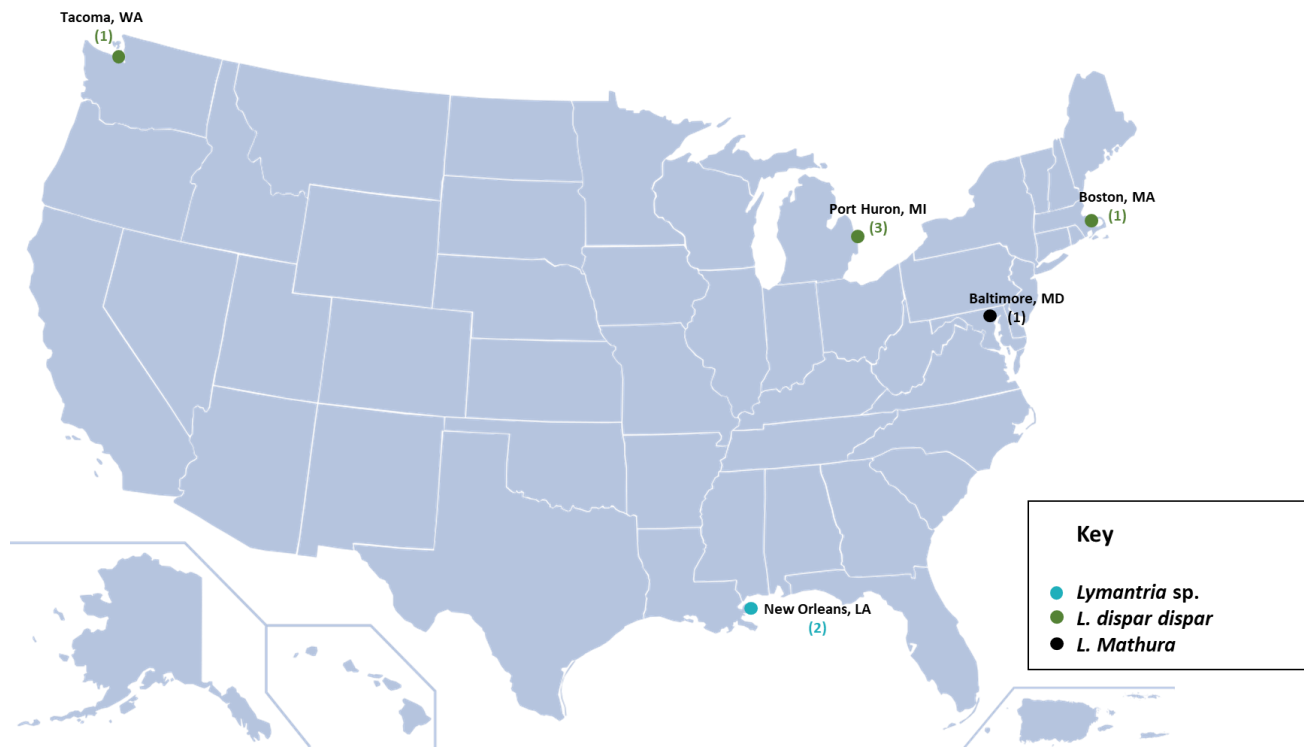
analyzes two genetic markers that vary between EGM and AGM. If a specimen of regulatory importance—trapped outside of the EGM quarantine or intercepted at a port—fails the Standard Diagnostic Assay, DNA barcoding is used to identify the species.

### 2018 Port Interception Results

In 2018, a total of 98 specimens were analyzed, representing 46 different interceptions made by Department of Homeland Security's Customs & Border Protection officials at ports of entry. Of the specimens analyzed, 79 specimens from 11 different ports of entry were identified as AGM (Figure 1). Additionally, five specimens were identified as EGM and one specimen intercepted in Baltimore, Maryland was identified as *Lymantria mathura* (Figure 2). Seven specimens were identified as species other than gypsy moth. The identities of three specimens remain unknown due to diagnostic assay failure. For an additional specimen, a DNA barcode was generated, however it did not match any reference barcodes in public databases.



**Figure 1.** In 2018, 79 specimens intercepted at various ports of entry (represented by red dots on the map) from June to October were identified as *Lymantria dispar asiatica/japonica* using molecular diagnostics.



**Figure 2.** In 2018, five specimens intercepted at ports of entry were determined to be *Lymantria dispar dispar*. One specimen, intercepted in Baltimore, MD was identified as *Lymantria mathura*. Two specimens, intercepted in New Orleans, LA were determined to be *Lymantria sp.* but could not be identified to species. Specimens were intercepted from February to September, 2018.

Two egg masses intercepted in New Orleans, Louisiana were identified as *Lymantria sp.* using DNA barcoding, each matching at a high percentage with reference barcodes for two different species of *Lymantria* (*L. scaeferi* and *L. apicebrunnea*). Adult *Lymantria* specimens collected in China during October, 2018 yielded barcodes that matched those of the two New Orleans egg masses. Morphological identification of the adult tissue (only wings and legs clipped from the specimen were available for analysis) suggested that the specimens may actually be *Lymantria xyliana*. Whole specimens from China will be collected in 2019 so that specimens can be dissected in order to make a final morphological identification.

### 2018 Domestic Survey Results

In 2018, a total of 3,241 specimens from 29 states were analyzed using the Standard Diagnostic Assay (Figure 3). Two AGM detections were confirmed: one specimen from Martha Lake, Washington and the other from Santa Cruz, California. Additional genetic analysis of the specimen found in Santa Cruz indicated that it shares an identical DNA sequence for an expanded mitochondrial region with another AGM specimen collected in the same location in August, 2017. This result suggests a close relationship—a shared matriarchal lineage—between the two moths.

Among the analyzed specimens, a total of 2,773 specimens were identified as EGM; 192 of these specimens were collected in areas outside of the EGM federal quarantine. Five specimens were identified as a species other than gypsy moth through DNA barcoding.

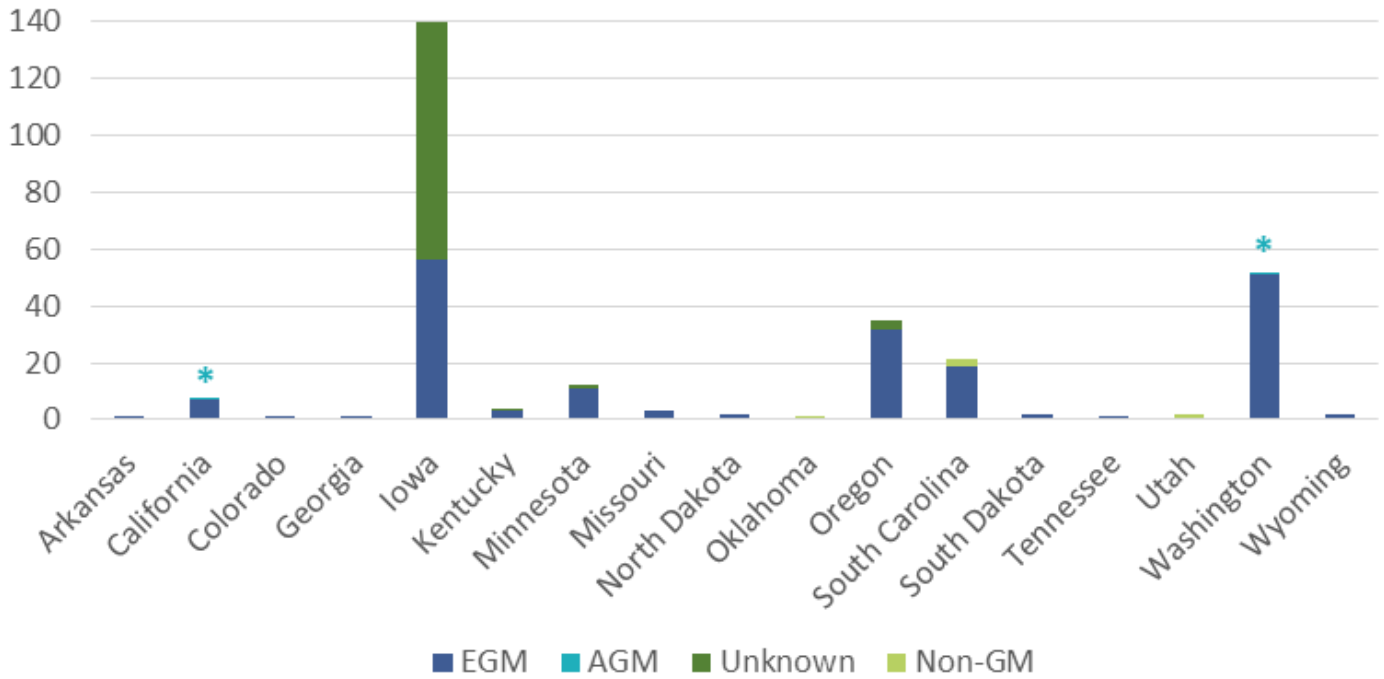
### Conclusion

In 2018, we observed a drastic increase in the number of AGM intercepted at ports of entry compared to recent years. Only one AGM egg mass was intercepted in 2016 (Long Beach, CA) and in 2017 no AGM were detected at ports of entry.

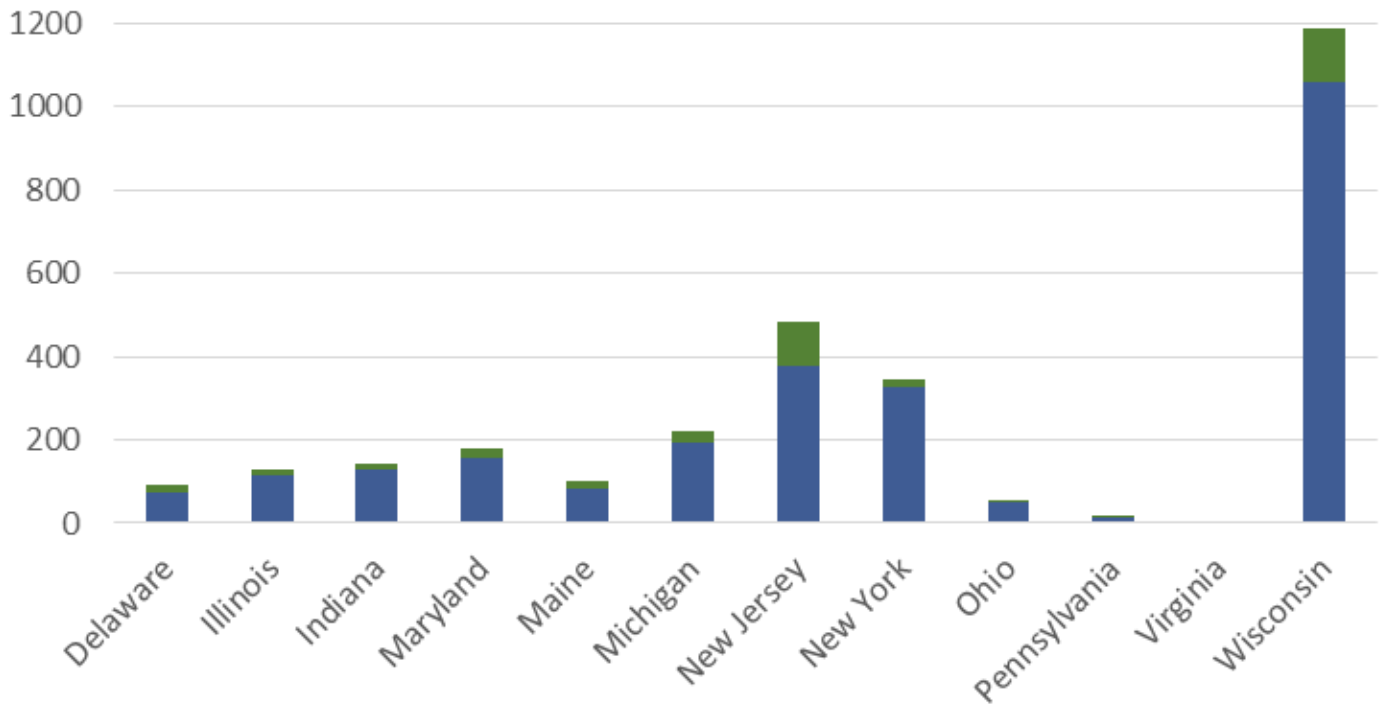
During this survey year a diagnostic processing priority system was implemented in which port interceptions and specimens found outside of the EGM quarantine were analyzed and results were reported within 24 hours of receiving the specimen. Traps from within the EGM quarantine were processed as they arrived at Otis. Using this streamlined process, we were able to report data back to participating surveying states by December of 2018—more than three months earlier than in past years. As the port and domestic Gypsy Moth Molecular Diagnostic Survey continues to provide valuable information to the Gypsy Moth Program, we aim to make improvements to increase the efficiency of the survey, as well as to improve our communication and relationships with stakeholders.



### Outside of Federal Quarantine



### Inside of Federal Quarantine



**Figure 3.** A total of 3,241 specimens from 29 states were analyzed for the 2018 Gypsy Moth Domestic Survey; 288 of these specimens were collected outside of the EGM federal quarantine. Two specimens—one from Santa Cruz, CA and the other from Martha Lake, WA—were determined to be *Lymantria dispar asiatica/japonica* using the Standard Diagnostic assay. A blue asterisk is used to bring attention to these two AGM detections in the graph.

## Development of a new generation of diagnostic tools for Asian gypsy moth

Yunke Wu<sup>1,2</sup>, Kendra Vieira<sup>1</sup>, Donald Stewart<sup>3</sup>, Michel Cusson<sup>3</sup>, Nevada Trepanowski<sup>1</sup>, and Marjorie Palmeri<sup>1,4</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>Cornell University, Department of Ecology and Evolutionary Biology, Ithaca, NY

<sup>3</sup>Canadian Forest Service Laurentian Forestry Centre, Natural Resources Canada, Quebec City, Quebec

<sup>4</sup>University of Massachusetts Amherst, Department of Environmental Conservation, Amherst, MA

### Introduction

The current Standard Gypsy Moth Diagnostic Assay functions to detect Asian gypsy moth, AGM, *Lymantria dispar asiatica/japonica*, in the U.S. and at ports of entry. Additionally, the assay distinguishes AGM from the other subspecies, European gypsy moth, EGM, *L. d. dispar*, which was introduced to North America ~150 years ago. The assay examines two genetic markers: a nuclear fragment designated as FS1 [1], and a mitochondrial marker associated with restriction enzymes that cleave DNA [2]. Both components utilize conventional PCR and have produced reliable data for over 20 years, but their efficiency has been challenged by the increasing number of samples that the Otis Laboratory receives for processing. Developing a new generation of diagnostic tools will provide an opportunity to increase the efficiency, specificity, and sensitivity of the Gypsy Moth Diagnostic Assay.

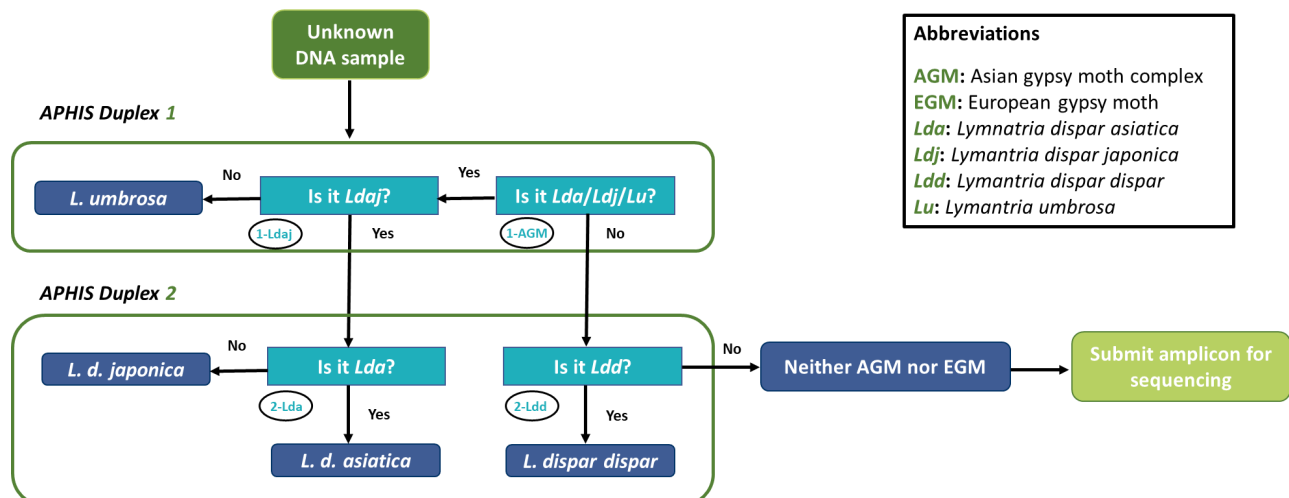
### Real-time PCR

The first tool utilizes a real-time PCR technique that was developed based on Stewart et al. 2016 [3]. Real-time PCR monitors the amplification of target DNA as it occurs as opposed to conventional PCR, which requires additional and post PCR agarose gel visualization. This protocol uses two duplex TaqMan assays to identify EGM, both subspecies of AGM (*L. d. asiatica* and *L. d. japonica*), and the Hokkaido gypsy moth, *L. umbrosa* (Figure 1). Nearly morphologically indistinguishable, *L. umbrosa* has been intercepted at U.S. ports in the past and is often confused with AGM. This assay would provide additional precision in the identification of *L. umbrosa*.

The biggest advantage of this new assay is the simultaneous identification of four different targets, which is not possible with the standard diagnostic assay. Additionally, it is more sensitive than the current method, requiring lower amounts of sample DNA for amplification. We will proceed to the validation step with known samples from our archive collected from Asia and intercepted at U.S. ports of entry. Preliminary validation data showed that among 93 samples of *L. d. asiatica* and *L. d. japonica*, the new assay correctly identified 90 as one of the two subspecies.

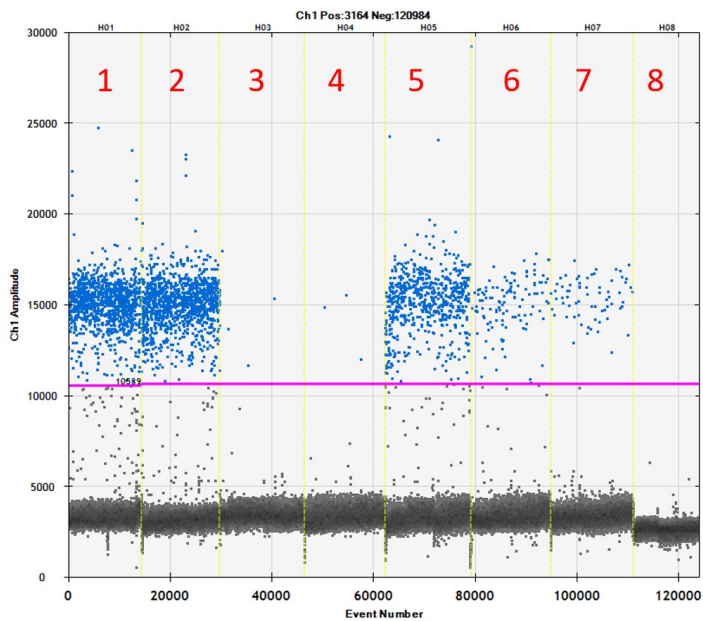
### Droplet digital PCR

The second tool utilizes droplet digital PCR technique (ddPCR), which partitions the reaction mix from conventional PCR into ~20,000 droplets, such that theoretically each droplet contains only one copy of target DNA. By isolating a single copy of DNA in each droplet the absolute amount of the target DNA can be quantified. Additionally, this partitioning allows for the detection of a specific target among nontarget DNA. Thus specimens can be mass-extracted and analyzed simultaneously in a single reaction tube [4, 5]. The ddPCR assay we are developing aims to detect AGM amongst the many EGM that are trapped for the Gypsy Moth Molecular Diagnostic Survey. The assay is based on the nuclear FS1 locus, which differs in amplified length between the Asian allele (312 base pairs) and North American allele (207 base pairs). Five sets of primers were designed to specifically amplify the Asian allele but not the North American allele. Due to the primers' high specificity, the presence of the Asian allele in the sample is determined when ddPCR returns a positive amplification, which is signaled through a SYBR Green dye.



**Figure 1.** Flow chart depicting the double-duplex TaqMan assay in real-time PCR. Individual primer pairs are named as: 1-AGM, 1-Ldj, 2-Ldd, and 2-Lda.

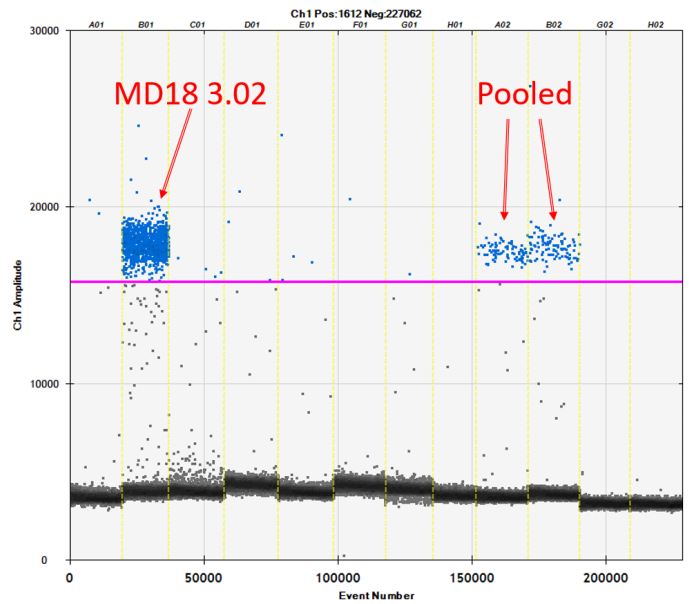
After a series of PCR parameter optimizations, we validated the new assay with a series of mixes and dilution using AGM and EGM reared from the quarantine facility. Two AGM and two EGM were used as standards and their DNA was normalized to 1 ng/ul, which roughly equals 1,000 Asian or North America copies per microliter. Then the AGM and EGM standards were mixed in 1:1, 1:9, and 1:24 ratios. The expected Asian allele copy number in those mixed samples should be 50%, 10%, and 4% of the AGM standards, respectively. The ddPCR estimations of Asian allele copy numbers were very close to expectations for both standards and mixed samples (Figure 2). The result showed that we can determine the presence of one AGM when mixed with 24 EGM.



**Figure 2.** Amplification plot from ddPCR assay for AGM and EGM standards and mixed and diluted samples. Blue dots are positive amplifications, and grey dots are negative amplifications. The order of samples are: 1, 2) AGM standards, 3, 4) EGM standards, 5) mixed 1:1, 6) mixed 1:9, 7) mixed 1:24, 8) negative control.

We further validated the ddPCR assay using domestic field collections from the FY18 Gypsy Moth Molecular Diagnostic Survey. Out of eight moths collected from Maryland, one specimen was known to possess one copy of the Asian allele per its genome.

The ddPCR successfully identified this moth during individual testing and also detected the presence of the Asian allele when DNA from all eight moths were pooled together (Figure 3). Those results support the batch processing capability.



**Figure 3.** Amplification plot from ddPCR assay for eight moths collected from Maryland. Blue dots are positive amplifications, and grey dots are negative amplifications. When the moth (MD18\_3.02) that possessed an Asian allele was pooled with the other seven specimens, the pooled DNA still produced a significant positive signal.

### Conclusions

We developed two new AGM diagnostic tools that can supplement and may eventually replace the current standard diagnostic assay. The real-time PCR assay provides opportunity to further distinguish between *L. d. asiatica* and *L. d. japonica* and identify *L. umbrosa*. We also showed that the ddPCR assay is sensitive enough to detect a single AGM in a mixed sample of 25 moths. In theory, we can possibly increase the mix ratio to 1:99. Both tools will boost our capacity to process more samples received from the annual gypsy moth survey with greater efficiency.

### References

- 1) Garner KJ, Slavicek JM. Identification and characterization of a RAPD-PCR marker for distinguishing Asian and North American gypsy moths. *Insect Mol Biol.* 1996; 5: 81–91.
- 2) Bogdanowicz SM, Wallner WE, Bell J, Odell TM, Harrison RG. Asian gypsy moths (Lepidoptera:
- 3) Lymantriinae) in North America: evidence from molecular data. *Ann Entomol Soc Am.* 1993; 86: 710–715.
- 4) Stewart D, Zahiri R, Djoumad A, Freschi L, Lamarche J, Holden D, et al. A Multi-Species TaqMan PCR Assay for the Identification of Asian Gypsy Moths (*Lymantria* spp.) and Other Invasive Lymantriines of Biosecurity Concern to North America. *PLoS ONE.* 2016; 11(8): e0160878.
- 5) Zink FA, Tembrock LR, Timm AE, Farris RE, Perera OP, Gilligan TM. A droplet digital PCR (ddPCR) assay to detect *Helicoverpa armigera* (Lepidoptera: Noctuidae) in bulk trap samples. *PLoS ONE.* 2017; 12: e0178704.
- 6) Zink FA, Tembrock LR, Timm AE, Gilligan TM. A ddPCR Assay for Identification of *Autographa gamma* (Noctuidae: Plusiinae) in Bulk Trap Samples. *J Econ Entomol.* 2017; 111: 1490-1495.

## Spatial genetic structure of Asian longhorned beetle in the United States and China

Yunke Wu<sup>1,2</sup>, Steven Bogdanowicz<sup>2</sup>, Kendra Vieira<sup>1</sup>, and Jose Andres<sup>2</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>Cornell University, Department of Ecology and Evolutionary Biology, Ithaca, NY

### Introduction

The Asian longhorned beetle, ALB, *Anoplophora glabripennis*, is an important pest insect endemic to China and Korea that was introduced into North America. Despite extensive eradication efforts, live beetles are still being found in the U.S. and Canada. Currently, there are three major extant U.S. populations: Bethel (Ohio), Long Island (New York), and Worcester (Massachusetts). Characterizing genetic structure among those populations will help us better understand the process of introduction. Carter et al. [1] previously analyzed genetic data of ALB collected from multiple North American locations between 1996 and 2007 but did not include Massachusetts and Ohio samples, which were only recently detected in 2008 and 2011, respectively. The genetic markers used in Carter et al. [1] were mitochondrial fragment and microsatellite loci, which resolved limited genetic patterns. Given our success of developing genome-wide markers using a modified double-digestion restriction associated DNA (ddRAD) sequencing approach for gypsy moth, we aim to develop similar amplicon data for ALB. The large number of markers generated through this approach are more likely to reveal subtle population structures.

### Methods and Results

We used ddRAD to identify genome fragments associated with microsatellite loci. Initial ddRAD library was generated through five representative specimens from each of the following locations: Boston (MA), Worcester (MA), Farmingdale (NY), Amityville (NY), and Bethel (OH).

We used Sbf1 and Msp1 restriction enzymes simultaneously with ligation adapter and targeted large fragments (size selected for 350–550 bp) for 2 × 300 bp paired-end sequencing on an Illumina Miseq platform at Cornell University's BioResource Center. We assembled over 26,500 genome fragments and discovered around 1,300 non-duplicated microsatellite loci that included dimers, trimers, and tetramers. Only tetramers were developed for the multiplex assay because it normally results in less noise in genotyping compared to dimers and trimers. Primers were developed for 76 tetramer loci, and 69 were used to genotype 174 specimens which were collected from Massachusetts, New York, Ohio, and Illinois and intercepted at U.S. ports of entry, as well as 50 specimens collected from two Chinese populations (Beijing and Yanchi, Ningxia). Raw genotypic data were trimmed to exclude microsatellite loci with > 20% missing

data and individuals with > 10% missing data. The final dataset includes 61 microsatellite loci and 199 individuals.

We used the discriminant analysis of principal components (DAPC) to characterize genetic clusters in sampled ALB. The optimal solution determined six groups (Figure 1), which demonstrated large genetic differentiation among the current three extant populations from Worcester (group 3), Long Island (group 4), and Bethel (group 5). The other three additional groups comprised of: the eradicated population from Staten Island, New York (group 1), some laboratory-reared ALB designated as the Worcester colony (group 2), and the eradicated Chicago population (group 6).

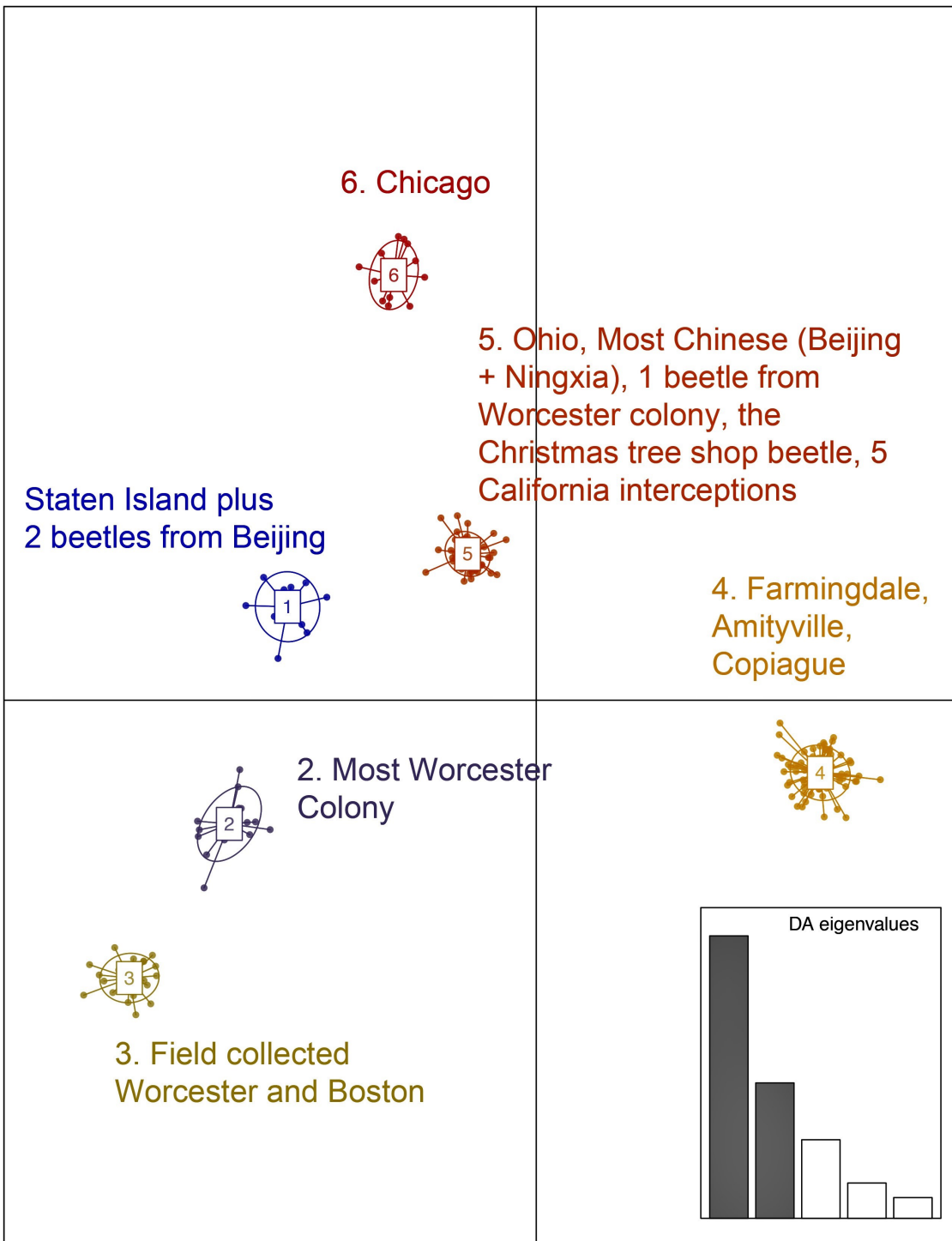
Despite the large geographic distance between the two Chinese populations (about 1,000 km), our data suggested a close relationship between them, which were clustered with the Bethel population in the U.S. (group 5). Five ALB larvae intercepted in California and one dead ALB specimen found at a Christmas Tree Shop in Michigan also fell into this group. This group of ALB could represent a widespread population in China (through human-aided movement) and is the major source of introduction in the U.S.

Additionally, we discovered that the Worcester colony maintained at Otis Laboratory differs from field collected Massachusetts samples. This result may indicate possible unrecorded hybridization from the Ohio colony, which is reared and maintained next to the Worcester colony. One Worcester colony specimen fell into the Ohio/China cluster, and the remaining 17 Worcester colony specimens were intermediate between field-collected Massachusetts samples and the Ohio/China cluster.

### Conclusions

We generated new next-generation sequencing data for ALB from all three extant U.S. populations and some Chinese samples. We demonstrated the lack of relatedness among the three U.S. populations, which would be the result of independent introductions. We also showed that extant populations are different from two eradicated populations from Staten Island and Chicago. The results provide important information about characteristics of the ALB invasion in the U.S. and its connection to source populations in China.





**Figure 1.** Discriminant analysis of principal components plot for U.S. and Chinese ALB based on 61 microsatellite loci. Each dot represents a single specimen. Ellipses show corresponding group.

**References**

1. Carter M, Smith M, Harrison RG. Genetic analyses of the Asian longhorned beetle (Coleoptera, Cerambycidae, *Anoplophora glabripennis*), in North America, Europe and Asia. *Biol Invasions*. 2010; 12(5): 1165–1182.

## European gypsy moth national risk assessment

Melissa Warden<sup>1</sup> and Gericke Cook<sup>2</sup>

<sup>1</sup>Otis Laboratory, USDA APHIS PPQ S&T CPHST, Buzzards Bay, MA

<sup>2</sup>USDA APHIS VS CEAH STAS RIRA, Fort Collins, CO

### Introduction

The European gypsy moth, EGM, *Lymantria dispar dispar*, risk assessment is a project with an annual deliverable that uses species distribution modeling to predict the arrival, establishment, and spread of gypsy moth in the United States. It started as a GIS analytical pilot project for the western region U.S. in 2011, then expanded to a national-level analysis in 2012. In 2013, APHIS partnered with the USDA Forest Service Forest Health Technology and Enterprise Team to benefit from their technical and modeling expertise to develop the 2014 risk assessment and survey sample design. This interagency collaboration elevated the past efforts by developing iterative regionalized statistical models, eliminating subjective data manipulation, for effective targeting of early detection [1].

The risk assessment is a consistent method of evaluating spread mechanisms and delivering an annual, nation-wide, continuous detection likelihood for EGM. The survey sample design uses the model output to allocate PPQ resources based on the estimated risk. The risk assessment and sample design provide information resources to field managers, and may be supplemented by additional local knowledge and data sources to meet goals in the Gypsy Moth Program.

### Methods and Results

The modeling framework was a species distribution model conducted in the open-source Software for Assisted Habitat Modeling (SAHM) [2]. Species distribution models identify areas on the landscape with site characteristics similar to locations where the pest has already been found; these areas are estimated to have a high likelihood of pest detection.

EGM detection data were provided from the Slow the Spread Foundation, APHIS PPQ, and state cooperators, to include trap locations and positive detections for 2008-2017. Locations and counts of positive detections were also obtained from the Otis Laboratory Gypsy Moth Molecular Diagnostic

Survey. Data were thinned so that all positive detections and all negative observations in the same year were at least five km apart in order to reduce spatial autocorrelation.

Site characteristics that were tested for relationships with EGM presence were mostly anthropogenic factors: USPS address forwards from within the federal EGM quarantine zone, population density, household income, road density, traffic volume, number of households with wood as primary fuel, and distance from rest stops, campgrounds, intermodal facilities, nurseries, sawmills, wood pallet manufacturers, military bases, universities, and weigh stations. Continuous dispersal kernels were also tested to address short to intermediate range spread, in the form of distance from a previous year population source ( $\geq$  three positive detections) and distance from the spread front (edge of EGM federal quarantine zone).

Because the importance of the different pathway predictors changed across space, the risk model consisted of four regionalized models: short range (within 200 km of the federal quarantine zone), intermediate range (200-500 km), long range ( $>$  500 km), and Maine (because the portion of Maine not under quarantine was isolated from the other model regions). The statistical modeling method used was a multivariate adaptive regression spline. The output from each regional model was a nationwide map of detection probability, and a maximum overlay of the regional models was computed to create the ensemble detection likelihood (Figure 1), although Maine was considered separately.

As a decision tool for gypsy moth program managers, stochastically distributed trapping locations for 2018 were determined by combining the detection likelihood with a host availability layer and a climate suitability layer (to mask out areas unsuitable for establishment). This sample design was used primarily by the national program to assist in evaluating allocation of program resources to states.

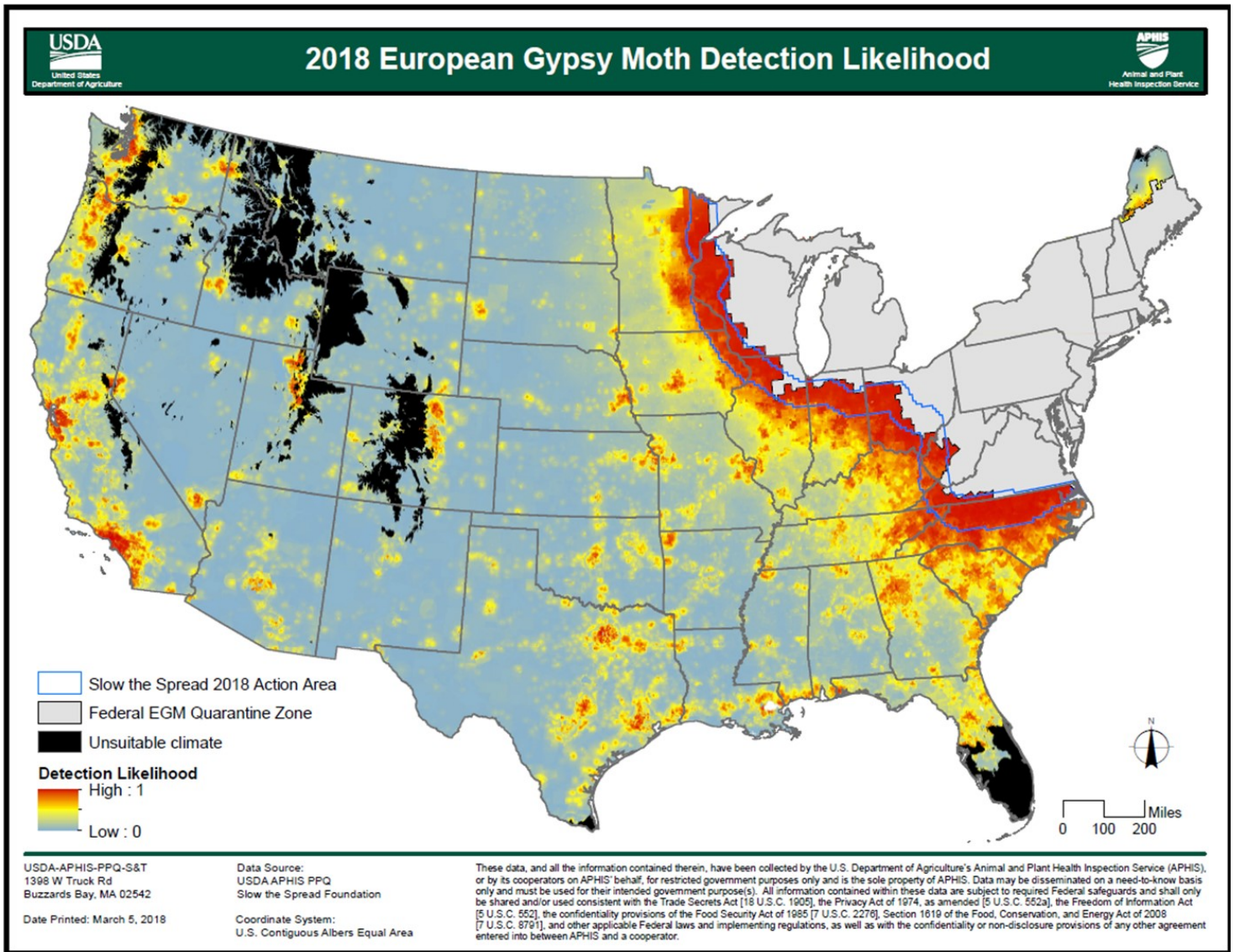


Figure 1. The 2018 European gypsy moth detection likelihood.

## References

- 1) Cook G, Jarnevich C, Warden M, Downing M, Withrow J, Leinwand, I. Iterative models for early detection of invasive species across spread pathways. *Forests*. 2019;10(2):108. <https://doi.org/10.3390/f10020108>
- 2) Morisette JT, Jarnevich CS, Holcombe TR, Talbert CB, Ignizio D, Talbert MK, Silva C, Koop D, Swanson A, Young NE. VisTrails SAHM: visualization and workflow management for species habitat modeling. *Ecography*. 2013;36:129–135. doi:10.1111/j.1600-0587.2012.07815.x

## A new record for *Tamarixia radiata* field cage production: 1 million wasps in 2018

Ruth Henderson<sup>1</sup>, Raju Pandey<sup>1</sup>, and Gregory Simmons<sup>2</sup>

<sup>1</sup>Citrus Research Board ACP Biocontrol Laboratory, Riverside, CA

<sup>2</sup>Otis Laboratory Salinas Field Station, USDA APHIS PPQ S&T CPHST, Salinas, CA

The Citrus Research Board (CRB) biological control group began methods-development to mass-produce *Tamarixia radiata* in large field cage insectaries in 2013 to contribute agents to the Southern California Asian citrus psyllid, ACP, *Diaphorina citri*, classical biological control program. In the first year, production reached only 120,000 wasps; however, production of *T. radiata* has increased annually as methods have been refined. In 2018, over 1.2 million *T. radiata* were produced in field cage insectaries. Over 1.1 million wasps were provided to CDFA for releases in Southern California, and an additional 9,200 were provided to biological control efforts in Yuma, AZ. Remaining wasps were used as additional starter material for field cages or were used in small-scale laboratory experiments. Overall, the program produced over 4.1 million *T. radiata* in 2018, 29% of which were produced by the CRB program (Table 1).

Construction of 2018 field cages began in April and ended in October, with a total of 41 cages build for *T. radiata* production. A total of 47 field cage harvests were conducted, including five cages which were constructed in 2017 and left up over winter. Four cages were constructed on grapefruit in a commercial orchard near Mentone, CA and the remaining cages in an experimental plot at Cal Poly Pomona. On average, 37,873

*T. radiata* were produced per cage. This is an increase of more than 14,000 wasp per cage over the 2017

average. The increase factor (number of wasps produced per wasp used in inoculation) was 26.9, an increase of 4 units over 2017 (Table 2). Production of *T. radiata* varied seasonally, with peak production coinciding with the hottest days, in August (Figure 1).

Around half of 2018 *T. radiata* production was from cages using *Citrus* rootstock varieties (Figure 2). Mexican lime, which was used as a host for experimental cages, provided a large proportion of *T. radiata* production this season (Figure 3). One of the Mexican lime cages produced 76,165 wasps in a single harvest, the largest single harvest on record. The success may be attributed to the new approach in pruning the trees. In the past Mexican lime trees were hedged on the outside, but trees used in 2018 were pruned severely, with most small shoots removed. Curry leaf, used in four harvested field cages this season, provided 11% of *T. radiata* production. The development of new methods for handling curry leaf as a host plant allows for greater production from this *Citrus* relative, and increased use of curry leaf as a host plant is planned in future seasons as it has been reported to be immune to Huanglong-bing or Citrus greening disease bacteria, *Candidatus Liberibacter asiaticus*.

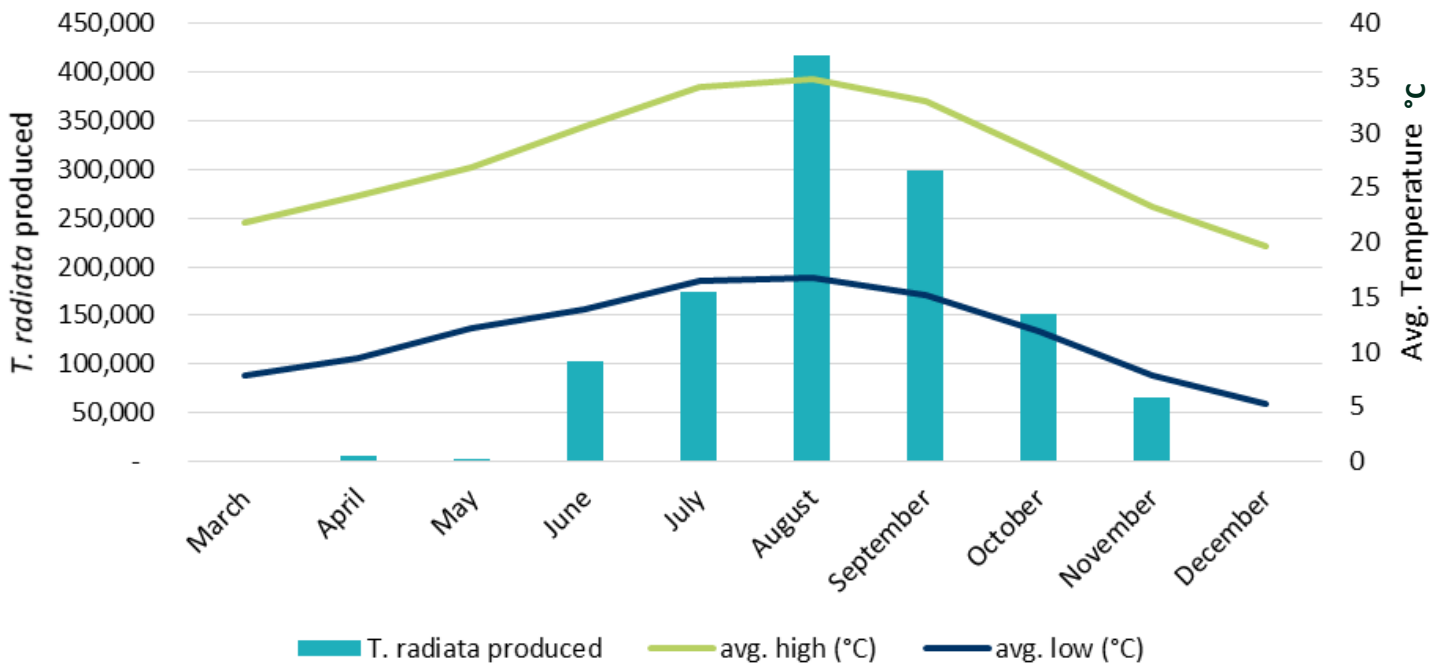
**Table 1.** Yearly *Tamarixia radiata* production by cooperating organizations. CRB totals include *T. radiata* produced in small laboratory or greenhouse dorms. Note: program contributions from FAR, a private insectary, ended in April, 2017.

Produced by	2013	2014	2015	2016	2017	2018	Total
CRB	119,142	240,456	510,897	607,019	878,827	1,219,037	3,575,378
FAR	–	137,524	265,961	147,850	205,383	–	756,718
UCR	161,057	296,881	165,445	523,015	650,748	400,212	2,197,358
CDFA	60,626	963,373	1,355,240	990,290	2,045,350	2,562,479	7,977,358
<b>Total</b>	<b>340,825</b>	<b>1,638,234</b>	<b>2,297,543</b>	<b>2,268,174</b>	<b>3,780,308</b>	<b>4,181,728</b>	<b>14,506,812</b>

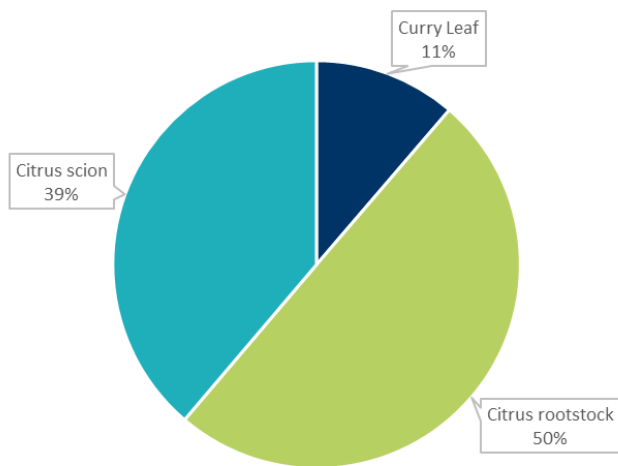
**Table 2.** A summary of *Tamarixia radiata* production figures, by year.

Year	No. Cages	No. harvests	Avg. TR per cage (±SE)	Max. TR per cage	Avg. Increase Factor (±SE)	Max. Increase Factor	Total TR Produced
2013	13	18	9,164(2,908)	32,418	11.6(3.3)	36.0	119,142
2014	35	39	5,830 (970)	22,520	8.4(1.3)	28.9	207,941
2015	46	58	10,411(1,308)	47,588	17.2(2.2)	66.5	480,702
2016	31	47	19,532(3,580)	82,730	25.2(3.9)	78.0	605,493
2017	32	54	23,738(2,682)	69,652	22.8(2.7)	77.8	861,492
2018	41	47	37,873(4,169)	87,434	26.9(2.7)	64.6	1,219,037

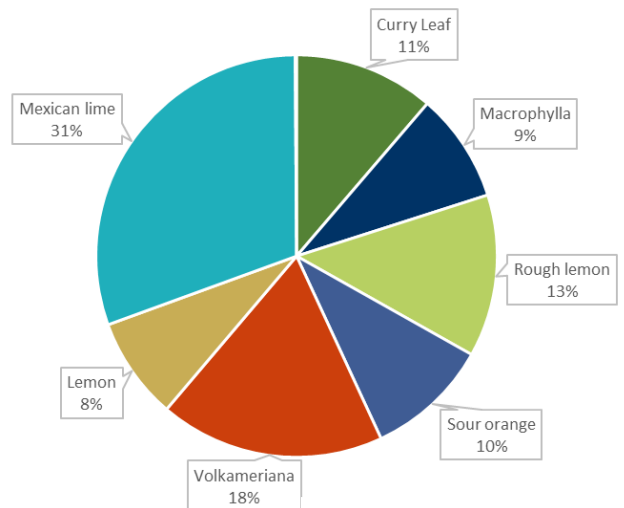




**Figure 1.** Monthly production of *T. radiata* from field cages (columns) in 2018 and monthly average high and low temperature (°C) in Riverside, CA (lines; data from [www.usclimatedata.com](http://www.usclimatedata.com)).



**Figure 2.** Percent of 2018 *T. radiata* production from cages using *Citrus* rootstock, *Citrus* scion, or curry leaf as host plants.



**Figure 3.** Breakdown of 2018 *Tamarixia radiata* production by host plant variety.

## Adjusting field cage inoculation rates of Asian citrus psyllid based on season

Ruth E. Henderson<sup>1</sup>, Raju Pandey<sup>1</sup>, and Gregory Simmons<sup>2</sup>

<sup>1</sup>Citrus Research Board ACP Biocontrol Laboratory, Riverside, CA

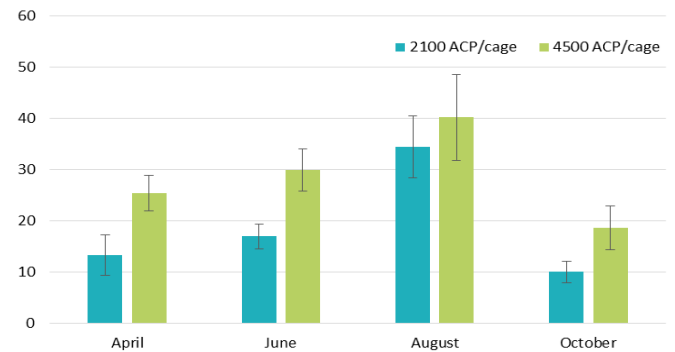
<sup>2</sup>Otis Laboratory Salinas Field Station, USDA APHIS PPQ S&T CPHST, Salinas, CA

Previously, the standard inoculation rate for Asian citrus psyllid, ACP, *Diaphorina citri*, has been 3,000 adults per large field cage. However, observations of the California Department of Food and Agriculture greenhouse system showed that during the warm summer month's small cages required smaller inoculations of ACP than in the colder winter months. Cages set up in the field during mid-summer and inoculated with only 2,000 ACP built up large ACP populations. These two observations suggest that larger ACP inoculations may help boost production of *T. radiata* during cooler seasons, while smaller inoculations may be sufficient during summer. This premise was first tested in field cages in 2017, with pairs of cages inoculated with either 3,000 or 1,500 ACP set up in April, June, and October. While cages inoculated with 3,000 ACP in June became heavily colonized—enough to be used for *T. radiata* production—none of the other cages built up sufficient enough host populations. In 2018, this experiment was revised.

Paired cages, built on 3-tree plots of Mexican lime, were set up in April, June, August, and October. Cages were inoculated with either 2,100 or 4,500 ACP seven days after pruning. When ACP nymphs reached 3<sup>rd</sup> and 4<sup>th</sup> instar, the number of flush points with ACP present and with ACP absent were counted. Four sample flush points per tree, one on each side of the tree, were then selected and the number of ACP nymphs present on each flush was counted. The percent of flush infested and the average number of ACP nymphs per flush were compared for each pair of cages.

In all cages inoculated with 4,500 ACP, 89% to 95% of flush became infested; however in cages inoculated with 2,100 ACP, infestation was below 90% in all cages except the one set up in August (Figure 1). The average number of nymphs found on infested flush varied throughout the seasons in cages receiving both high and low doses of ACP at inoculation.

There were significantly more nymphs per flush in cages inoculated with 4,500 ACP April (T-test;  $P = 0.02$ ) and June (T-test;  $P < 0.01$ ) than in those inoculated with 2,100 ACP, but nymphs per flush did not differ statistically between paired cages set up in August and October (Figure 2).

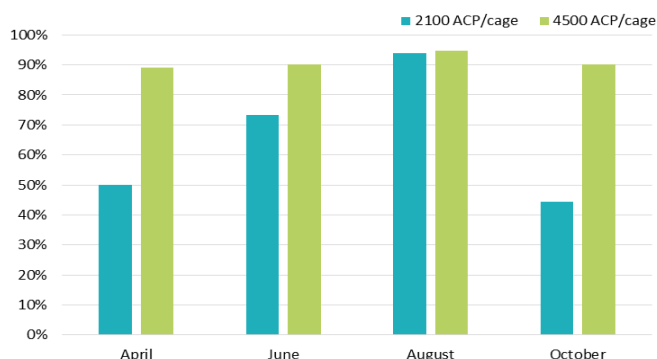


**Figure 2.** Average number of ACP nymphs present on sampled ACP-infested flush. Error bars represent standard error. \*Indicates a significant difference (T-test,  $P < 0.05$ ) between cages inoculated with 2,100 and 4,500 ACP.

Often, cages set up in spring, before mid-May, do not build a large enough ACP population to support *T. radiata* production within one generation. Similarly, cages set up in October or later are often not usable before the following spring. In this study, the April cage inoculated with 4,500 ACP became populated with enough nymphs to use for *T. radiata* production and went on to produce an above-average number of wasps. Cages inoculated at both the 2,100 and 4,500 ACP rates were used to produce *T. radiata* in June and August, though in both cases yield was higher in cages inoculated at the higher rate. Neither cage set up in October was used for *T. radiata* production (Table 1). This suggests that *T. radiata* production cages set up in spring and early summer can be boosted by larger ACP inoculations, allowing them to produce *T. radiata* in numbers comparable to those produced by cages set up later in summer.

**Table 1.** Production of *T. radiata* from cages with two rates of ACP inoculation.

Setup Month	ACP inoculated	<i>T. radiata</i> produced
April	2,100	0
	4,500	56,082
June	2,100	8,348
	4,500	22,248
August	2,100	53,370
	4,500	76,145
October	2,100	0
	4,500	0



**Figure 1.** Percent of flush with ACP eggs and/or nymphs present.

## Phenology of Asian citrus psyllid in the Southwestern Colorado Desert of Arizona

Francesc Gomez-Marco<sup>1</sup>, Marco Gebiola<sup>1</sup>, Richard Stouthamer<sup>1</sup>, and Gregory Simmons<sup>2</sup>

<sup>1</sup>University of California, Department of Entomology, Riverside, CA  
<sup>2</sup>Otis Laboratory Salinas Field Station, USDA APHIS PPQ S&T CPHST, Salinas, CA

### Introduction

Asian citrus psyllid, ACP, *Diaphorina citri*, was first detected in San Luis in October, 2009 without signs of Huanglongbing (HLB) infection [1]. Following this discovery, a part of Yuma County and an area of 32 km around Lake Havasu City in Mohave County were immediately placed under Federal quarantine for ACP by the United States Department of Agriculture. As temperature drives insect development and behavior, the phenology of its life stages can be predicted by degree-days (DDs) accumulation [2,3]. These predictions may be used to control pest populations by targeting specific life stages, thus informing management decisions. Predicting the pest phenology with DD models integrated on larger databases could improve pest management programs across different geographic regions, and biocontrol programs in particular, as they may be more affected by climate patterns and how pest population dynamics evolve over time/temperature [3]. To evaluate the long-term establishment of *T. radiata* and its impacts on ACP populations in this biological control program, there is a need to understand the fluctuations in population dynamics of ACP. Modeling the different ACP life stages most vulnerable to parasitism or predation will result in a more precise targeting of the pest in different locations [3]. By providing a tool to assist with the development of sustainable management practices, these models based on temperature may then help halt the spread of ACP in Arizona.

### Methods

We reconstructed the phenology of ACP in urban areas in the lower Colorado Desert of Arizona for three years (2015-2017). Surveys of ACP were carried out as described in [4]. The percentage of total cumulative ACP eggs, 1<sup>st</sup> to 3<sup>rd</sup> and 4<sup>th</sup> to 5<sup>th</sup> instar nymphs, and adult days were calculated over each of the three sampling years and then regressed against accumulated daily temperatures to model the population dynamics of each development stage in relation to degree days.

### Results

We showed that each year all ACP stages (eggs, nymphs, and adults) were present at two different time periods, from early spring until early summer and in smaller numbers in fall, whereas populations of ACP were extremely low—near the limit of detection—during the three months of summer (Figure 1). This disappearance may be due to the high summer temperatures typical of the surveyed areas. We also confirmed that the cumulative temporal populations of ACP life stages infesting citrus

can be predicted using deterministic DD models. The DD predictions obtained in our models were similar to those obtained by [3] for urban citrus trees in Southern California, but with two main differences.

First, the exponential increase phase of ACP populations occurred earlier in Arizona (April-May) than in Southern California (May-July). As a result, the maximum populations were reached earlier in Arizona than in Southern California. However, ACP populations do not increase during summer in the Arizonan desert, as in Southern California. Secondly, urban citrus grown in Arizona supports lower year-round ACP densities compared to urban citrus grown in Southern California. Two factors may explain this difference: 1) ACP populations are extremely reduced in the heat of Western Arizona summers because thermal maximum temperature for development is exceeded; and 2) Citrus have reduced flushing periods under these summer weather conditions.

High temperatures can also affect the flying behavior of ACP [5], hence ACP adults will have limited time during the day to search for other suitable host in a desert urban environment, where the citrus trees are scattered. An additional factor to consider is that exposure to high temperatures, between 40 and 42°C, for a minimum of 48 hours, was sufficient to significantly reduce or eliminate *Candidatus Liberibacter asiaticus* entirely in HLB-affected citrus seedlings [6]. These temperatures are easily reached and surpassed in our study sites during summer. However, the curing effect of high temperatures under field conditions remains to be evaluated. Decisions to manage ACP on urban citrus trees will depend on these factors and the availability of control tools.

It is likely that only urban trees nearest to commercial citrus production areas would need management, as ACP dispersal is limited within a year. These factors reduce the risk of ACP spreading, and the possible harboring of HLB in urban citrus in the Western Arizona desert is a minor threat for commercial citrus compared to Southern California. In fact it is not clear if these ACP populations, if unmanaged, represent a significant risk to commercial citrus production. This will depend on how close specific ACP infested urban citrus trees are to production areas, their quality as hosts, and if a given year's environmental conditions favor ACP population growth.

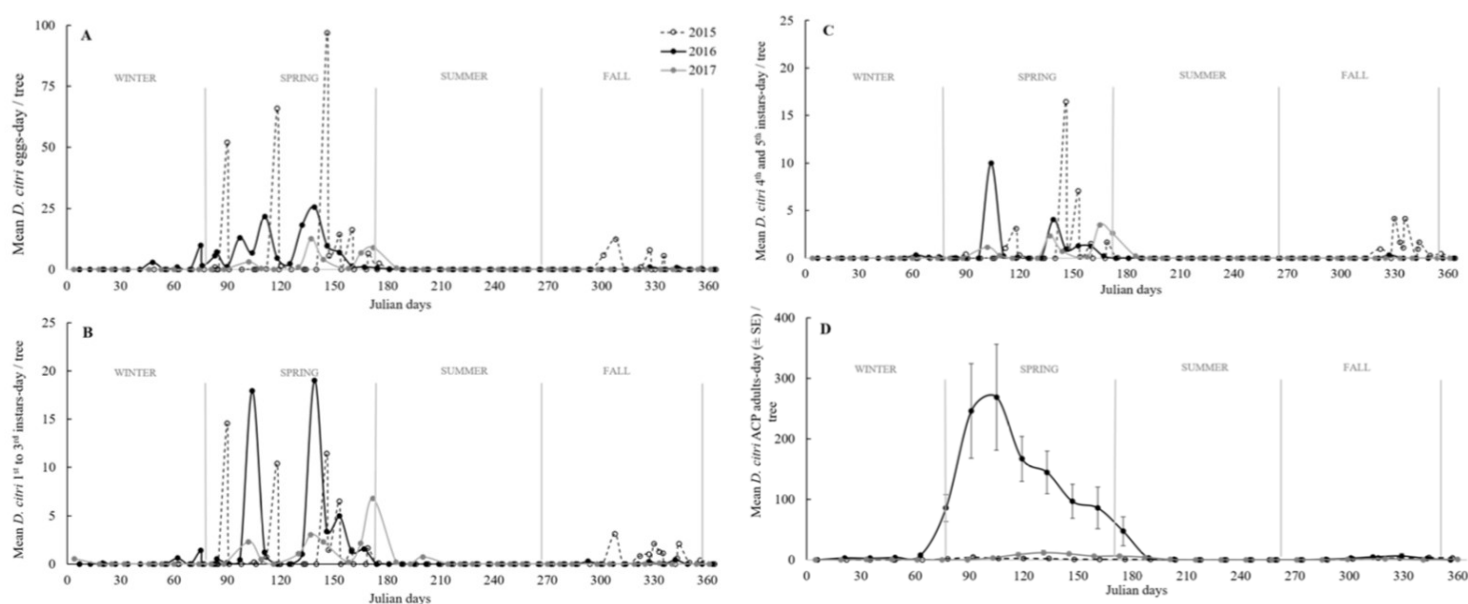
The rate of increase of ACP population growth may be dependent on the number of adults that overwinter to start the next generation and these in turn are dependent on how many ACP adults survive the previous summer. We have shown that summer temperatures in this area cause a severe population bottleneck for ACP populations persisting into the fall. Understanding how and where ACP survive during the summer months may be important for managing the risk of ACP near commercial citrus. While the numbers are extremely low, some adults can be found during the summer months.

How they survive is uncertain, though it is known in the native range of ACP in Pakistan that adults manage to survive on citrus where summer temperature can peak at 45°C [7]. There is evidence that there may be a period of heat acclimation that al-

lows adult ACP to better adapt for high summer heat survival [8]. This may be occurring in the dry hot desert citrus production areas of Arizona, California and Mexico, along with ACP adults finding cooler places with suitable micro-climatic niches within these areas where there may be higher survival. Future surveys will be conducted in such areas to possibly identify niches where ACP may survive.

### Conclusion

A manuscript [9] about ACP phenology in Arizona was published in June of 2019. A new survey protocol has been implemented since March 2018 aimed at linking the phenology of ACP with the phenology of its Citrus hosts by determining the presence or absence of flush growth using a steel hoop “ring method”.



**Figure 1.** Population dynamics of means of cumulative insect-days of ACP A) eggs, B) 1st to 3rd instar nymphs, C) 4th and 5th instar nymphs pooled across trees with ACP presence of any stages on each year per site and D) adults. Immature ACP stages data were obtained from visual surveys from 2015 throughout 2017, whereas adult data were obtained from yellow sticky traps placed on citrus trees throughout the same time.

### References

- 1) Mead F, Fasulo T. Asian Citrus Psyllid, *Diaphorina citri* Kuwayama (Insecta: Hemiptera: Psyllidae). FDACS/DPI Entomol. 2010;Circular, 180.
- 2) Gómez-Marco F, Tena A, Jaques JA, García AU. Early arrival of predators controls *Aphis spiraecola* colonies in citrus clementines. J. Pest. Sci. 2016;89:69-79.
- 3) Milosavljević I, Amrich R, Strode V, Hoddle MS. Modeling the phenology of Asian citrus psyllid (Hemiptera: Liviidae) in urban Southern California: effects of environment, habitat, and natural enemies. Environ. Entomol. 2018;47: 233-243.
- 4) Gebiola M, Gómez-Marco F, Stouthamer R, Simmons GS. Phenology and classical biological control of Asian citrus psyllid in Arizona and preliminary evaluation of ACP predators. Otis laboratory annual report. 2017;114-115.
- 5) Tomaseto AF, Miranda MP, Moral RA, de Lara IAR, Fereres A, Lopes JRS. Environmental conditions for *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) take-off. J. Appl. Entomol. 2018;142:104-113.
- 6) Hoffman MT, Doud MS, Williams L, Zhang M-Q, Ding F, Stover E, Hall D, Zhang S, Jones L, Gooch M, Fleites L, Dixon W, Gabriel D, Duan Y-P. Heat treatment eliminates '*Candidatus Liberibacter asiaticus*' from infected citrus trees under controlled conditions. Phytopathology 2013;103:15-22.
- 7) Khan SZ, Arif MJ, Hoddle CD, Hoddle MS. Phenology of Asian citrus psyllid (Hemiptera: Liviidae) and associated parasitoids on two species of citrus, kinnow mandarin and sweet orange, in Punjab Pakistan. Environ. Entomol. 2014;43:1145-1156.
- 8) Hall DG, Hentz MG. Asian citrus psyllid (Hemiptera: Liviidae) tolerance to heat. Ann. Entomol. Soc. Am. 2014;107:641-649.
- 9) Gomez-Marco F, Gebiola M, Baker BG, Stouthamer R, Simmons GS. Impact of the Temperature on the Phenology of *Diaphorina citri* (Hemiptera: Liviidae) and on the Establishment of *Tamarixia radiata* (Hymenoptera: Eulophidae) in Urban Areas in the Lower Colorado Desert in Arizona. Environmental entomology. 2019 May 14;48(3):514-23.



## *Tamarixia radiata* inoculation rate for its mass production in greenhouses

Raju Pandey<sup>1</sup>, Ruth Henderson<sup>1</sup>, Gregory Simmons<sup>2</sup>, Judith Herreid<sup>3</sup>, and David Morgan<sup>3</sup>

<sup>1</sup>Citrus Research Board ACP Biocontrol Laboratory, Riverside, CA

<sup>2</sup>Otis Laboratory Salinas Field Station, USDA APHIS PPQ S&T CPHST, Salinas, CA

<sup>3</sup>Mt. Rubidoux Field Station, CDFA, Riverside, CA

### Introduction

*Tamarixia radiata* is currently being mass-reared and re-released as part of a multi-agency effort to establish classical biological control of the invasive Asian citrus psyllid, ACP, *Diaphorina citri*, in California and Arizona. Asian citrus psyllid is the vector of the *Candidatus Liberibacter asiaticus* bacterium, the putative causal agent of the citrus greening disease, HLB, huanglongbing. The three most important biological factors impacting the *T. radiata* production system are: 1) identification of suitable host plants, 2) identification of the appropriate ACP density to inoculate a cage, and 3) identification of suitable densities of *Tamarixia radiata* wasps to inoculate a cage. Large curry leaf plants have previously been found to be the best variety of host plant, and inoculating cages with 300-400 ACP adults has been found to produce the greatest number of ACP nymphs to be used for *T. radiata* production.

*Tamarixia radiata* is a solitary parasitoid; only one wasp can successfully develop on each ACP nymph. However, the female wasps also host feed to fulfil their protein need for egg maturation. Inoculating a cage with too many wasps can lead to low *T. radiata* production due to higher rate of killing by host feeding or superparasitism and probing that can lead to host mortality. On the other hand, inoculating with too few *T. radiata* in a cage when there is high density of ACP nymphs is likely to lead to many nymphs escaping parasitization. Using an inoculation rate of 100 *T. radiata* wasps per cage has been the recent standard practice; however, *T. radiata* production is not consistent across cages.

Studies were conducted at California Department of Food and Agriculture greenhouses at Mt. Rubidoux during summer 2017 to determine the *T. radiata* inoculation density required for mass production.

### Methods

ACP were reared following the standard practice of using 300 adults per cage within BugDorm-2400 Insect Rearing Tents (W30 x D30 x H47 in.) with nine curry leaf plants in each cage. *Tamarixia radiata* were inoculated when ACP nymphs reached 3<sup>rd</sup> or 4<sup>th</sup> instar. Subsequent inoculations were carried out two days after the previous inoculation. Our previous study showed that a higher inoculation rate (125 wasps/cage) was less productive than a lower inoculation rate. Fifty female wasps per cage, the lowest rate tested, produced the highest number of *T. radiata*. In this year's study, *T. radiata* inoculation rates tested ranged from 25 to 100 females per cage, as either one or two split releases (Tables 1 and 2). Of the nine replications completed, cages from three replications produced much higher numbers of *T. radiata*. This is presumably largely attributed to the availability of better quality plants for those setups, leading to better ACP nymph production. Therefore, the data from these three replications were analyzed separately (Table 2). Parameters compared included total *T. radiata* production per cage, net *T. radiata* produced per cage (= total *T. radiata* produced – number of initial *T. radiata* used) and *T. radiata* increase rate (= Total *T. radiata* produced per cage/Total *T. radiata* used per cage).

**Table 1.** Number of *T. radiata* produced with various inoculation rates and timing (mean of six replications).

<i>T. radiata</i> used			<i>T. radiata</i> produced		
1 <sup>st</sup>	2 <sup>nd</sup>	Total	Total	Net	X-factor
25	0	25	849a	824a	34.0a
50	0	50	698a	648a	14.0b
25	50	75	874a	799a	11.6b
50	50	100	867a	767a	8.7b
F <sub>3,15</sub> ; (P)			0.75; (0.54)	0.71; 0.560	48.1; (<0.001)

**Table 2.** Number of *T. radiata* produced with various inoculation rate and timing (mean of three replications).

<i>T. radiata</i> used			<i>T. radiata</i> produced		
1 <sup>st</sup>	2 <sup>nd</sup>	Total	Total	Net	X-factor
25	0	25	1,542a	1,517a	61.7a
50	0	50	2,363a	2,313a	47.3ab
25	50	75	3,155a	3,080a	42.1ab
50	50	100	2,286a	2,186a	22.9b
$F_{3,6}$ (P)			1.46 (0.32)	1.38 (0.34)	4.63 (0.05)

## Results

Under a low productivity regime, the greatest net *T. radiata* production was from cages that received a single inoculation of 25 *T. radiata* females, followed by cages that received a split release of 25, followed by 50. A single release of 50 female wasps per cage resulted in the lowest net *T. radiata* production; however, none of the treatments were statistically different from each other. The *T. radiata* increase factor was highest (34.0) when only 25 wasps were used for inoculation, which was significantly different from the increase factor for rest of the treatments (Table 1).

Under high productivity regime, the highest number of *T. radiata* was produced when 75 females were used to inoculate the cages followed by inoculation with 50 and 100 wasps. Cages receiving only 25 wasps produced the least number of *T. radiata*. However, there were not significant differences between the various inoculation densities tested. The highest multiplication factor of 61.7 was obtained for 25 wasps per cage followed by 50 and 75 wasps per cage. These three treatments were not significantly different from each other.

## Conclusions

Our field cage studies suggest that releasing one *T. radiata* to every 50 ACP nymphs has improved *T. radiata* production; however, it is extremely difficult to quantitatively estimate the nymph population within the greenhouse cages prior to *T. radiata* inoculation due to the small size of nymphs as well as their tendency to stay concealed deep into the feathery parts of the plants.

Under a low productivity regime (possibly due to plant quality and ACP densities; Table 1), a low inoculation density of *T. radiata* (25 wasps per cage) produced better results. Under a moderate production regime (data from previous year; not shown here), 50 *T. radiata* per cage resulted in better production. Under a higher productivity regime, (better plant

quality with higher ACP infestation on the plants; Table 2) inoculating a cage with 75 *T. radiata* female wasps resulted in better production. Based on these results and experience gained from previous years' studies, the number of *T. radiata* to be used for inoculating a cage should vary based on the ACP densities. A guideline was developed to estimate ACP densities qualitatively to help *T. radiata* inoculations.

**Very high:** Most stems with clusters of nymphs that have migrated well below the tip of the stems, and a lot of honeydew. Release 30 (25F + 5M) *T. radiata* at first release and 60 (50F + 10M) *T. radiata* at second release.

**High:** Some stems with ACP nymphs, leaves with prolific honeydew, honeydew accumulated on lower leaves or cage floor. Release 30 (25F + 5M) *T. radiata* at first release and 30 (25F + 5M) *T. radiata* as second release.

**Medium:** Leaves with moderate density on leaf rachis, few nymphs on stems if the apical leaves are dead. Release 30 (25F + 5M) *T. radiata* at first release only.

**Low:** Sparse ACP nymphs on all plants, no nymphs on stem, and few leaves with moderate nymph density. Release 15 (10F + 5M) *T. radiata* at first release only.

**Very low:** Very sparse ACP nymphs, no ACP nymphs on more than half of the plants. Do not release any *T. radiata*.

The recommended timing for the first inoculation is when the nymphs begin to move to the leaf base and/or stem tip, usually 3<sup>rd</sup> instar nymphs. The second inoculation should be made two to three days after first inoculation, when ACP nymphs are mostly 4<sup>th</sup> and 5<sup>th</sup> instar. Inoculation with a fewer *T. radiata* was found more advantageous than inoculations with too many *T. radiata*, which could be related to ACP mortality due to host feeding by *T. radiata*.

## Alternative collection methods for *Tamarixia radiata*

Raju Pandey<sup>1</sup>, Ruth Henderson<sup>1</sup>, Gregory Simmons<sup>2</sup>, Judith Herreid<sup>3</sup>, and David Morgan<sup>3</sup>

<sup>1</sup>Citrus Research Board ACP Biocontrol Laboratory, Riverside, CA

<sup>2</sup>Otis Laboratory Salinas Field Station, USDA APHIS PPQ S&T CPHST, Salinas, CA

<sup>3</sup>Mt. Rubidoux Field Station, CDFA, Riverside, CA

### Introduction

The parasitoid *Tamarixia radiata* is being mass-produced for use as a biological control agent of the Asian citrus psyllid, ACP, *Diaphorina citri*, vector of the citrus greening disease, HLB, huanglongbing. The California Department of Food and Agriculture is the primary *T. radiata* producer in the state of California. Collection of adult *T. radiata* is the most time consuming and labor-intensive step in the mass production process. Studies were conducted to find efficient and effective methods for collection of *T. radiata*.

### Light activated collection

Because *T. radiata* exhibits positive phototaxis behavior, it is collected efficiently in a Florida mass production facility by attaching a clear jar to the top of the cage. When the cages are covered with black cloth *T. radiata* move upward into the jar. This method was adapted for the Mt. Rubidoux greenhouse production system using dome-shaped bug dorms. The system utilizes natural sunlight to attract the wasps to the jar, but a major drawback to this system was the need for additional labor to cover and uncover cages.

There is also an increased risk of *T. radiata* mortality due to elevated temperatures beneath the black cloth, especially during the hot summer months. The possibility of using artificial light sources to aid *T. radiata* collection outside of the greenhouse was explored. Experiments were conducted to compare efficacy of three types of light sources: LED halo, pink LED, and white LED.

The three styles of LED light sources were placed at a distance from the end of the collection jars to preventing excessive heating. The light sources were tested 25 times using curry leaf plant clippings laden with ACP mummies that were transferred to bug dorms on three-tier shelves. Black plastic curtains were used to cover the whole shelving to create darkness except at the jar attachment site. Each day, the curtains were closed, jars were attached, and lights were placed behind the jar and turned on for a duration of one hour. After an hour, jars were removed and capped. Immediately after, any remaining *T. radiata* in each dorm were collected by aspiration. The percent of *T. radiata* collected in the jars was calculated and compared for each light source. The mean *T. radiata* collection by the tested light sources was below 50% (Table 1), and the majority of wasps had to be collected manually.

**Table 1.** The percent of *T. radiata* collected in jars illuminated by three different light sources.

Light Type	Percent <i>T. radiata</i> collected
LED Halo	45.9 (±6.3)
Pink LED	47.9 (±5.7)
White LED	46.0 (±4.9)

The additional effort required to set up the jar and light was not justified by the amount of *T. radiata* collected.

### Plant clipping

Unlike many endoparasitoids where the parasitoid pupae are completely encased within host tissue that can easily be removed from the substrate, *T. radiata* is an ectoparasitoid and thus lies under the host remains rather than inside of them. Pupae of *T. radiata* are protected beneath the mummified ACP exoskeleton that is tightly attached to the plant surface. Therefore, removing ACP mummies from plant tissue is not a practical method and can lead to desiccation of the pupae.

Experience from the field cage insectaries has shown that clipping the plant tissue laden with mummified ACP and placing it in emergence cages allows for efficient collection of *T. radiata*. This could potentially shorten the production cycle in greenhouse cages and increase overall production of *Tamarixia*. The study tested the plant clipping method along with other potential methods detailed below:

- Collection by vacuum aspirator (standard practice)
- Collection by a jar attached to the top of the cage, followed by vacuum aspirator
- Plant clipping transferred to emergence cages when *T. radiata* begins to emerge followed by vacuum aspirator collection
- Modified leaf blower/vacuum device for mass collection

The cages assigned to the jar collection method produced the highest numbers of *T. radiata* followed by leaf blower collection cages. The leaf blower method was the quickest way to collect the wasps. One problem associated with leaf blower collection was contamination with ACP adults. Cages assigned to manual collection either with or without clipping the plant tips produced fewer *T. radiata* (Table 2). The study was completed after two replications.

**Table 2.** Number of *T. radiata* collected per cage for various treatments.

Collection methods	Setup 1	Setup 2
Vacuum aspirator	232	932
Jar method	917	981
Tip clippings	640	487
Leaf blower	606	690

### Conclusions

Efficiency of *T. radiata* collection in the jars backed by supplemental light was poor. Early pruning and transfer of plant tips to emergence cages was less productive. Modified leaf blower method was the quickest method of collection. ACP contamination with *T. radiata* may be addressed by using a selective screen on the nozzle that permits *T. radiata* but restricts ACP.

## Trends in insecticide use in California nurseries: Mining the Pesticide User Reports from CDFA for possible effects of LBAM control

H. Alejandro Merchan<sup>1,2</sup>, Gregory S. Simmons<sup>1</sup>

<sup>1</sup>Otis Laboratory Salinas Field Station, USDA APHIS PPQ S&T CPHST, Salinas, CA

<sup>2</sup>North Carolina State University, Department of Entomology, Raleigh, NC

### Introduction

The light brown apple moth, LBAM, *Epiphyas postvittana*, was first reported in Berkeley, California, in 2007 [1]. Over the last 10 years, there has been significant spread in coastal California areas and parts of Northern California; many counties were added to the LBAM regulated areas within the quarantine [2]. Regulatory actions and control measures were applied to many LBAM host commodities. Over time, many of these commodities were exempted from interstate regulation for movement within the United States based on the pest mitigations provided through industry standards of production, harvesting, and packaging practices for each of the exempted commodities, which greatly reduces the risk of further movement on these commodities.

Currently, nursery operation and shipping outside of LBAM regulated areas is still a significant risk pathway for the invasive moth. Due to this risk, under state and federal regulations, the California Department of Food and Agriculture (CDFA) and USDA regulate wholesale ornamental plant nurseries within the LBAM regulated areas. Nurseries within the regulated area are subjected to monthly inspections and required to stay free of LBAM, which often requires frequent pesticide treatments of entire nurseries. Also every shipment outside of the regulated area must be inspected prior to shipment, treated with an approved insecticide, and inspected again prior to shipment. To help better understand and to reduce the regulatory burden on nurseries due to this invasive pest, we have developed a tool to help analyze the impacts of LBAM on nursery production and to determine the potential costs of the program. Using publically available data from CAL-Department of Pesticide Regulation we analyzed at the county level the changes in pesticide use over time for LBAM recommended treatments [3]. Our goal is show the patterns of changes in pesticide use as the invasion progressed to test if LBAM control has produced significant changes in insecticide use in California nurseries. A secondary goal is to develop tools to analyze the efficacy and impacts or large scale regulatory programs.

### Data Mining

CDFA publishes yearly summaries of the pesticide use in California and all data is made available for download at [ftp://transfer.cdpr.ca.gov/pub/outgoing/pur\\_archives](ftp://transfer.cdpr.ca.gov/pub/outgoing/pur_archives). At writing, 2016 was the last year that had publicly available data. We downloaded all the PUR files from 2004 to 2016 and developed our own query tools using the Julia programming language. We decided to develop our own tools as a proof of concept for the use of Julia for data mining tasks, and also because we wanted more control over the type of queries and data cleaning we could perform. Julia is a new programming language, with an easy and readable syntax that produces and compiles fast and efficient code, making it a good candidate to develop our scripts. Julia is free and open source, and all scripts are available from the authors upon request.

### Analysis

We defined a quarantine county as any that has an active LBAM quarantine in the CDFA map for March, 2019 [3]. This assumption clearly overestimates the effect of the quarantine since most counties only have a small area of quarantine, but we count every nursery in the county as quarantined against LBAM. We did this to study the effect at a county level, but we're trying to refine the geographical scale at which we can analyze the insecticide use. We plan to keep developing scripts so we can analyze the data at different geographical levels.

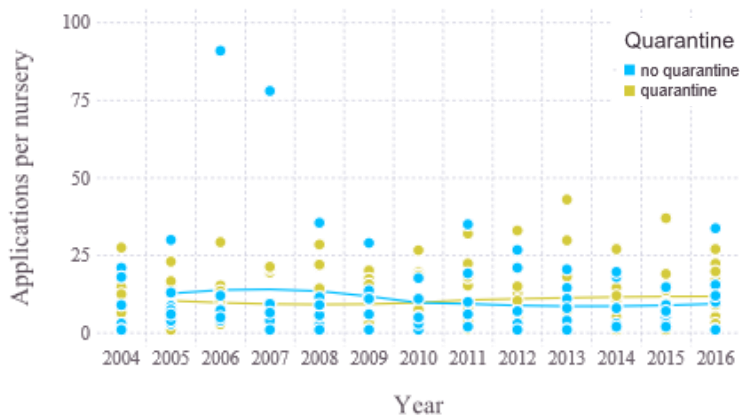
Insecticide applications per grower were calculated by dividing all available applications of each insecticide in each county by the number of unique grower ID's. We wanted to control for differences in the size of the industry in different counties, because nurseries are distributed mostly in coastal counties in California. We also did the same transformation for the amount of pounds of active ingredient applied for each grower each year. We present the results for each insecticide.



**Results**

*Lambda-cyhalothrin use*

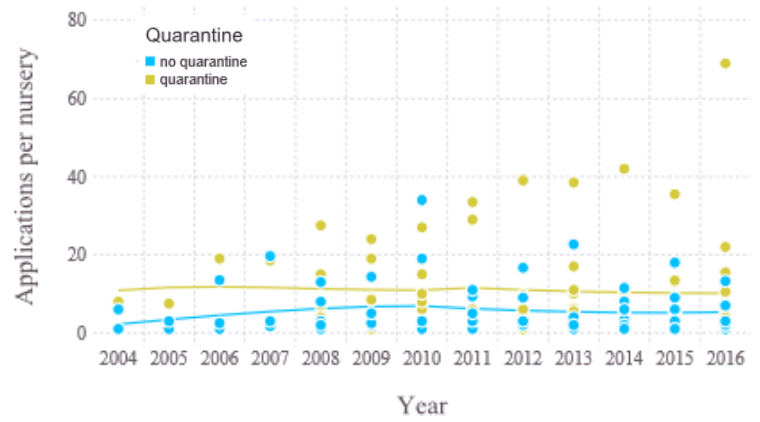
The pyrethroid insecticide lambda-cyhalothrin has been very effective in LBAM control in our tests and it is commonly used in the nursery industry. We determined that since 2011 the use of lambda-cyhalothrin is higher in quarantine than in non-quarantine counties, after removing outliers (Figure 1). Despite this higher use, the number of pounds of active ingredient applied per nursery remain higher in non-quarantine counties. This suggests that treatments with lambda-cyhalothrin are probably applied in small areas, consistent with the requirements of the LBAM control program.



**Figure 1.** Number of insecticide applications per nursery per year applied in counties belonging to the LBAM quarantine area vs. counties not in the quarantine area for lambda-cyhalothrin. Lines represent a loess linear regression line for each year for quarantine (green) and non-quarantine counties (blue).

*Methoxyfenozide use*

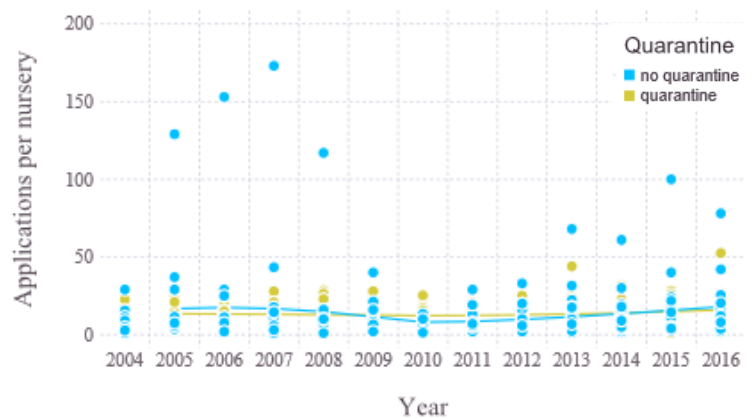
Methoxyfenozide is an insect-growth regulator that is also very effective against LBAM. The use of this insecticide has been increasing in nurseries in California since 2004, but its growth has been steeper in counties that are part of the LBAM quarantine area (Figure 2). As with lambda-cyhalothrin, the higher number of applications within the quarantine area does not correspond to a higher number of pounds of active ingredient applied in the nurseries, suggesting again that while use is more frequent, it is in small quantities. This observation is probably related to shipments of nursery stock outside of the control area.



**Figure 2.** Number of insecticide applications per nursery per year applied in the counties belonging to the LBAM quarantine area vs. counties not in the quarantine area for methoxyfenozide. Lines represent a loess linear regression line for each year for quarantine (green) and non-quarantine counties (blue).

*Spinosad use*

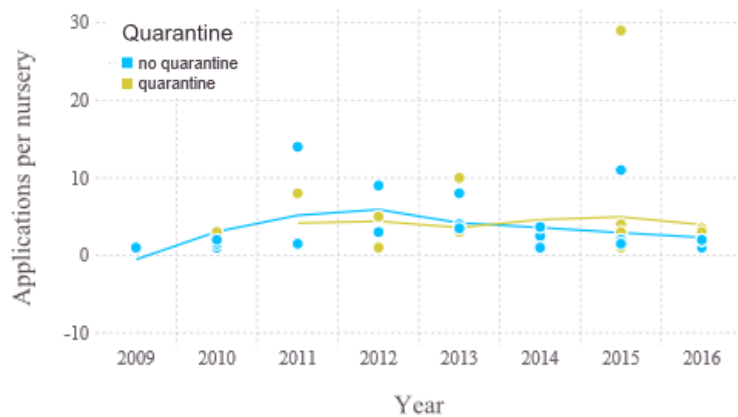
The use of spinosad has been very prevalent in nurseries in California and trends in use between the quarantine area and the non-quarantine area are not very different (Figure 3). The pounds of spinosad applied in the non-quarantine area has been more variable, but has grown since 2010. In the quarantine area, the pounds of spinosad applied appear to be fairly stable. Spinosad is an organic insecticide, the information is probably being captured from a wider variety of nurseries that will not use any of the other conventional insecticides, which may also explain its higher usage rate.



**Figure 3.** Number of insecticide applications per nursery per year applied in counties belonging to the LBAM quarantine area vs. counties not in the quarantine area for spinosad. Lines represent a loess linear regression line for each year for quarantine (green) and non-quarantine counties (blue).

### Chlorantraniliprole use

This insecticide is fairly new in the market and started being reported in nurseries in 2009. The number of applications remains low and there are no differences between the quarantine and the non-quarantine counties (Figure 4). The pounds of active ingredient applied for this chemical is slightly higher in the non-quarantine area, but that difference has diminished in 2016—the last year we have data. We expect an increase in the use of this insecticide in the coming years due to its efficacy against Lepidoptera larvae and its good environmental profile.



**Figure 4.** Number of insecticide applications per nursery per year applied in the counties belonging to the LBAM quarantine area vs. counties not in the quarantine area for chlorantraniliprole. Lines represent a loess linear regression line for each year for quarantine (orange) and non-quarantine counties (blue).

### Conclusions

- Data for insecticide use collected by the California Department of Pesticide Regulation (CDPR) is very valuable to study trends in insecticide use at different geographical locations in California. However, we need to improve the tools to interact and analyze the data. The scripts developed in this project are publicly available upon request and we will continue our analysis of these data.
- Nurseries are exposed to a wide variety of pest pressures and it is hard to correlate any particular insecticide application with the LBAM program. Regardless, this project can provide trends and help determine if there have been obvious changes in insecticide use.
- Increases in the number of applications of lambda-cyhalothrin and methoxyfenozide suggest that the program has indeed caused changes in insecticide use in the nursery industry.
- We will analyze the data from other insecticides that are labeled for use against leafrollers because nurseries can use those to maintain their plants free of the pest. We will also analyze the data at a more granular scale—in a way that allows us to improve the spatial correlation between the data and the quarantine area.

### References

- 1) Brown JW, Epstein ME, Gilligan TM, Passoa SC, Powell JA. Biology, Identification, and History of the Light Brown Apple Moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae: Archipini) in California: An Example of the Importance of Local Faunal Surveys to Document the Establishment of Exotic Insects, *American Entomologist*. 2010; 56(1): 34–43
- 2) Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. *N Engl J Med*. 2002 Jul 25;347(4):284-7.
- 3) Light Brown Apple Moth (LBAM). Map of quarantine boundaries. March 21, 2019. [https://maps.cdfa.ca.gov/QuarantineBoundaries/LBAM/LBAM\\_PQM\\_State\\_Overview.pdf](https://maps.cdfa.ca.gov/QuarantineBoundaries/LBAM/LBAM_PQM_State_Overview.pdf)
- 4) Light Brown Apple moth (LBAM). Approved pesticide treatments. February 16, 2018. <https://phpps.cdfa.ca.gov/PE/InteriorExclusion/PDF/LBAMApprovedTreatments.pdf>

## Biological control of Asian citrus psyllid in Arizona using the parasitoid *Tamarixia radiata*

Marco Gebiola<sup>1</sup>, Francesc Gomez-Marco<sup>1</sup>, Bobby Baker<sup>2</sup>, Paul Rungman-Jones<sup>1</sup>, Richard Stouthamer<sup>1</sup> and Gregory Simmons<sup>3</sup>

<sup>1</sup>Department of Entomology, University of California, Riverside, CA

<sup>2</sup>USDA APHIS PPQ FO, Yuma, AZ

<sup>3</sup>Otis Laboratory Salinas Field Station, USDA APHIS PPQ S&T CPHST, Salinas, CA

### Introduction

*Tamarixia radiata*, a parasitoid of Asian citrus psyllid, ACP, *Diaphorina citri*, was introduced from the native area, Punjab Pakistan in 2011 and has been mass reared at the Insectary & Quarantine facility of the University of California, Riverside (UCR) in order to develop a biological control program against this pest in Southern California and Arizona [1]. Since late 2013 a large effort has been put in place aimed at establishing *T. radiata* in Yuma county and Lake Havasu City in Mohave County. More recently, *T. radiata* have also been released in Ajo, AZ—a small town 100 miles east of Yuma—due to an outbreak of ACP during the summer of 2018.

### Releases of *T. radiata* in Yuma and Mohave county, AZ

*T. radiata* were produced and released as detailed in [1]. A total of 434,031 *T. radiata* have been released in Yuma and Lake Havasu City from December, 2013 to December, 2018 (Table 1).

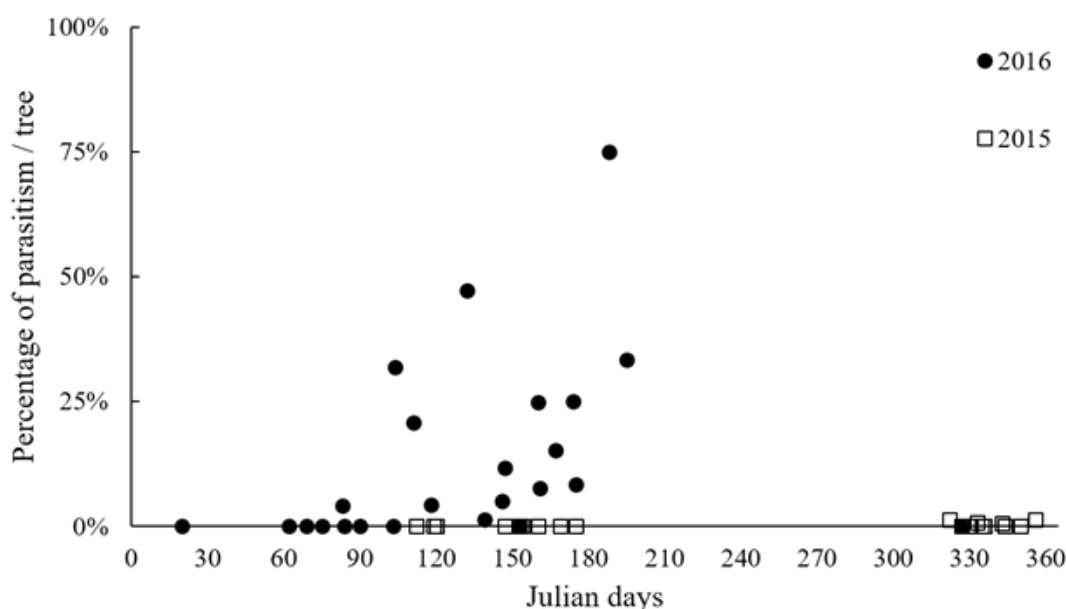
The mortality of *T. radiata* prior releases (shipping mortality) was always below 7%. Parasitism rates by *T. radiata* differed by year, peaking on average at around 50% from April to June of 2016, the year ACP was most abundant.

**Table 1.** Number of *Tamarixia radiata* released in Arizona until December, 2018.

Year	Agents released
<i>Yuma and Lake Havasu city</i>	
2013	1,820
2014	54,320
2015	80,132
2016	96,502
2017	102,179
2018	99,078
<i>Ajo</i>	
2018	15,800

The average parasitism in 2016 was  $12.52 \pm 3.79\%$  (SE) when the suitable hosts (4<sup>th</sup> and 5<sup>th</sup> instar ACP nymphs) were present, from beginning of March to end of June (Figure 1). However, parasitism rates were low in 2015 (~1.4% in fall) (Figure 1) and no parasitism was recorded in 2017 or 2018.

These observations are consistent with previous studies that show *T. radiata* parasitism rates varying significantly depending on geographic area, season, and availability of appropriate life stages for parasitism [2-6]. The parasitoid was recovered at least once at 23 of 32 survey sites, and in one in-



**Figure 1.** Percentage parasitism for *T. radiata* attacking ACP nymphs on citrus trees from Arizona (Yuma County and Mohave County) in 2015 and 2016 on trees with suitable ACP nymphal stages are shown.

stance at a location ~24 km away from the closest release site. Sequencing of a fragment of the mitochondrial gene COI confirmed that *T. radiata* recovered at this non-release and non-survey site were indeed from UCR (Figure 2).

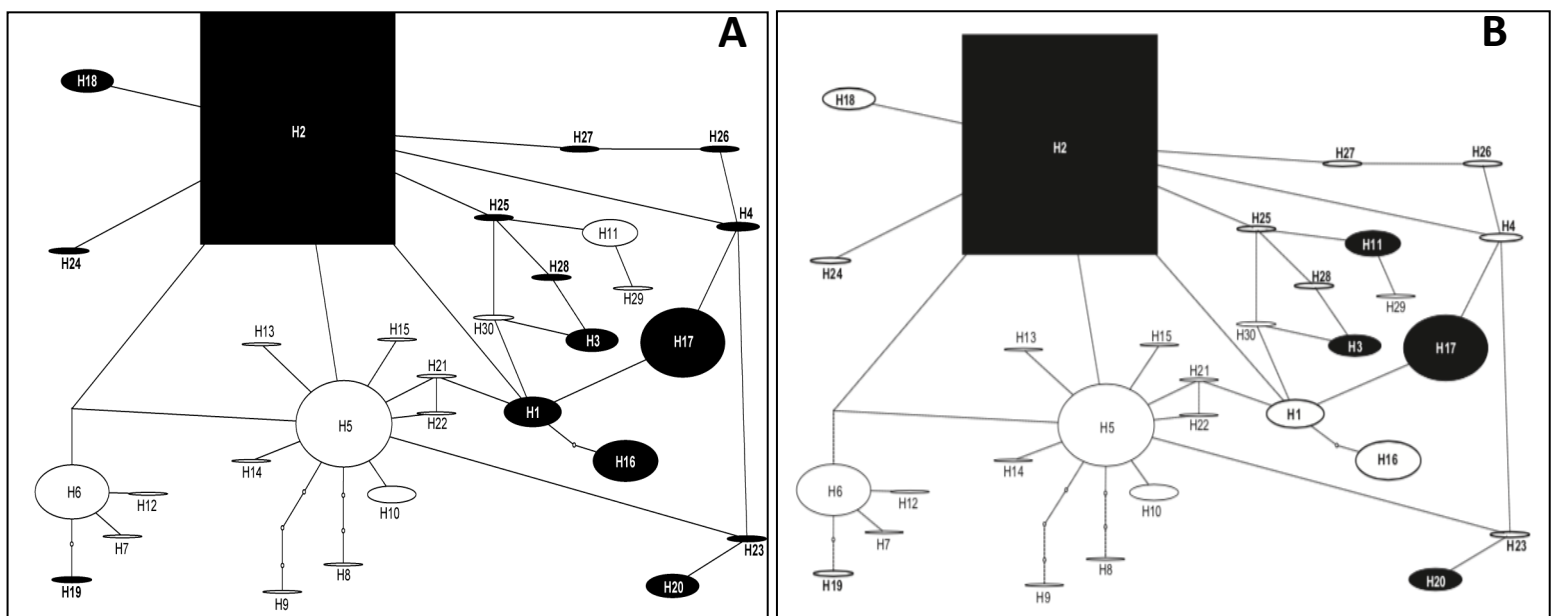
Because the survey sites were distant from the release sites, the 2016 recoveries suggest that *T. radiata* established for part of the year (or one winter), but it is unclear if they are capable of permanent establishment, as shown by the absence of parasitism throughout 2017 and 2018. Failure to permanently establish would be consistent with the fact that ACP may not be able to reproduce under the summer desert temperatures [7], thus depriving the specific parasitoid *T. radiata* of hosts for a prolonged period. Continued surveys of these areas in subsequent years after discontinuation of releases may confirm either a lack of permanent establishment or if *T. radiata* is present in numbers so low as to often be undetectable. One way of assessing establishment may be the use of yellow sticky traps once releases of *T. radiata* end, or placing traps at increasing distances from release sites (>5 miles) while releases are ongoing.

From March, 2018 to March, 2019 the feasibility of using yellow sticky traps to monitor *T. radiata* was tested by screening traps that are used to monitor ACP. Eleven *T. radiata* specimens were trapped at nine of the 32 trapping sites in the four working zones (three in Yuma County, one in Lake Havasu City) (Table 2). Sequencing of COI confirmed that

trapped *T. radiata* were indeed all of UCR origin. Four haplotypes were recovered out of nine successfully sequenced specimens, indicating a good genetic diversity of the mass reared biocontrol agents (Figure 2, Table 2).

### Releases of *T. radiata* in Ajo, AZ

Sampling of ACP in Ajo, AZ by personnel at USDA-APHIS in Phoenix during the 2018 summer revealed an outbreak of ACP at this location where ACP had been observed for a few years at 6-month sampling intervals, always at low population density. Therefore it was decided to release *T. radiata* in Ajo as well, in an attempt to drastically reduce the level of ACP infestation. A total of 15,800 *T. radiata* have been released from September to December, 2018. Ajo is located at higher elevation than Yuma and its cooler summer temperatures that allow ACP to thrive might also allow *T. radiata* to establish permanently in the area. On December 13, 2018—two months after the latest release of *T. radiata*—two ACP mummies enclosing healthy *T. radiata* pupae were collected in a backyard. Sequencing of one of these pupae recovered a COI haplotype (H11) reared and released by UCR (Figure 2B). In Ajo, ACP was also monitored by using yellow sticky traps. Increasing attraction of ACP to traps using a commercial lure containing methyl salicylate—a volatile emitted by HLB-infected citrus plants to attract ACP and infect adults [8]—was tested by placing a bag of lure next to three of the six traps deployed around town.



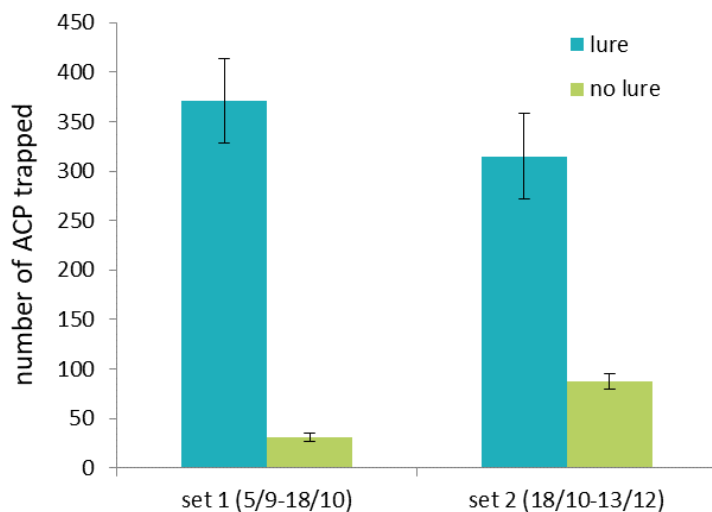
**Figure 2.** Pakistani COI haplotypes reared and released by UCR (A) and recovered in Arizona in 2018 (B), in black circles. All haplotypes in panel B were recovered in Yuma county and Mohave county, except for haplotype 11 recovered in Ajo.



**Table 2.** *Tamarixia radiata* trapped in Yuma County and Mohave County, Arizona from March, 2018 to March, 2019 and their COI haplotypes.

Trap #	Site	Zone	Locality	Service date	<i>T. radiata</i>	COI Haplotype
ACP15LHBC28002	BC28	4	Lake Havasu City	06/01/18	1	H2
ACP15SLBC13002	BC13	2	San Luis	08/03/18	1	H3
ACP15YBC21002	BC21	3	Fortuna Foothills	08/17/18	1	N/A
ACP15SBC8001	BC8	1	Yuma/Somerton	09/28/18	1	H3
ACP15SLBC11001	BC11	2	San Luis	09/28/18	2	H3
ACP14YBC4002	BC4	3	Fortuna Foothills	10/26/18	1	H17
ACP15YBC6002	BC6	1	Yuma/Somerton	10/26/18	1	H2
ACP15SLBC18001	BC18	2	San Luis	10/26/18	1	H20
ACP15YBC20001	BC20	3	Fortuna Foothills	10/26/18	1	N/A
ACP15YBC20002	BC20	3	Fortuna Foothills	10/26/18	1	H3

The first set of traps was put in place on September 5 and serviced on October 18, when a second set was deployed and then serviced on December 13. Traps with lures caught a significantly higher number of ACP than traps without lures (Figure 3).

**Figure 3.** Number of ACP trapped in Ajo from September to December, 2018 with and without methyl salicylate lures.

## Conclusions

Our work has shown that the *Tamarixia radiata* biotypes produced by the UCR production facility in and released in Yuma AZ has been recovered and caused high mortality of ACP during the year when ACP was most abundant. We have also documented extensive movement of *T. radiata* which suggests it has become established for at least part of the study period. Due to the high summer temperatures in Western Arizona that affect ACP reproduction, it is unclear whether permanent establishment of *T. radiata* has been achieved.

The program will stop releasing *T. radiata* in Yuma in 2019 so that monitoring can occur in subsequent years. Future releases of *T. radiata* will take place in other areas of the state where ACP detections have occurred in effort to widely establish the new parasitoid across all areas of Arizona where ACP is found.

## References:

- 1) Soper A, Hare J, Withman T, Manzo D, Stouthamer R. Preserving genetic variability in mass-rearing *Tamarixia radiata* to control ACP. *Citrograph*. 2014 Fall;5(4):58–63.
- 2) Tsai JH, Wang JJ, Liu YH. Seasonal abundance of the Asian citrus psyllid, *Diaphorina citri* (Homoptera: Psyllidae) in southern Florida. *Fla. Entomol.* 2002;85:446–451.
- 3) Michaud JP. Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in central Florida. *Biol. Control.* 2004;29:260–269.
- 4) Qureshi JA, Stansly PA. Exclusion techniques reveal significant biotic mortality suffered by Asian citrus psyllid *Diaphorina citri* (Hemiptera: Psyllidae) populations in Florida citrus. *Biol. Control.* 2009;50:129–136.
- 5) Kistner EJ, Amrich R, Castillo M, Strode V, Hoddle MS. Phenology of Asian citrus psyllid (Hemiptera: Liviidae), with special reference to biological control by *Tamarixia radiata*, in the residential landscape of Southern California. *J. Econ. Entomol.* 2016;109:1047–1057.
- 6) Milosavljević I, Amrich R, Strode V, Hoddle MS. Modeling the phenology of Asian citrus psyllid (Hemiptera: Liviidae) in urban Southern California: effects of environment, habitat, and natural enemies. *Environ. Entomol.* 2018;47: 233–243.
- 7) Gomez-Marco F, Gebiola M, Baker BG, Stouthamer R, Simmons GS. Impact of the Temperature on the Phenology of *Diaphorina citri* (Hemiptera: Liviidae) and on the Establishment of *Tamarixia radiata* (Hymenoptera: Eulophidae) in Urban Areas in the Lower Colorado Desert in Arizona. *Environmental entomology.* 2019 May 14;48(3):514–23.
- 8) Mann RS, Ali JG, Hermann SL, Tiwari S, Pelz-Stelinski KS, Alborn HT, Stelinski LL. Induced release of a plant-defense volatile “deceptively” attracts insect vectors to plants infected with a bacterial pathogen. *PLoS Pathog.* 2012;8(3):e1002610.

## 2018 Publications

*Otis Laboratory members are indicated in bold*

- Arthur FH, Ghimire MN, **Myers SW**, Phillips TW. Evaluation of Pyrethroid Insecticides and Insect Growth Regulators Applied to Different Surfaces for Control of *Trogoderma granarium* (Coleoptera: Dermestidae) the Khapra Beetle. *J Econ Entomol.* 2018;111(2):612-19.
- **Cooperband MF**, **Mack R**, Spichiger SE. Chipping to Destroy Egg Masses of the Spotted Lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae). *J Insect Sci.* 2018;18(3):7.
- Kashian DM, Bauer LS, Spei BA, Duan JJ, **Gould JR**. Potential Impacts of Emerald Ash Borer Biocontrol on Ash Health and Recovery in Southern Michigan. *Forests.* 2018;9(6):296.
- Gebiola M, Gomez-Marco F, **Simmons GS**, Stouthamer R. Effect of host feeding on life history traits of *Tamarixia radiata*, parasitoid of the Asian citrus psyllid, *Diaphorina citri*. *BioControl.* 2018;63(6):763-71.
- **Gould JR**, Aflague B, **Murphy TC**, McCartin L, Elkinton JS, Rim K, et al. Collecting Nontarget Wood-Boring Insects for Host-Specificity Testing of Natural Enemies of *Cerambycids*: A Case Study of *Dastarcus helophoroides* (Coleoptera: Bothrideridae), a Parasitoid of the Asian Longhorned Beetle (Coleoptera: Cerambycidae). *Environ Entomol.* 2018;47(6):1440-50.
- Hallman GJ, Wang L, Maxwell F, Barrios CEC, Vreysen MJB, **Myers SW**. Comparison of Three Populations of *Bactrocera dorsalis* for Efficacy of Vapor Heat Treatment in Mangoes. *Florida Entomologist.* 2018;101(2):219-22.
- Duan JJ, Bauer LS, Van Driesche RG, **Gould JR**. Progress and Challenges of Protecting North American Ash Trees from the Emerald Ash Borer Using Biological Control. *Forests.* 2018;9(3):142.
- Menocal O, Cruz LF, Kendra PE, Crane JH, **Cooperband MF**, Ploetz RC, et al. *Xyleborus bispinatus* Reared on Artificial Media in the Presence or Absence of the Laurel Wilt Pathogen (*Raffaelea lauricola*). *Insects.* 2018;9(1):30.
- Jones MI, **Gould JR**, **Warden ML**, Fierkea MK. Dispersal of emerald ash borer (Coleoptera: Buprestidae) parasitoids along an ash corridor in western New York. *Biological Control.* 2018;128:94-101.
- **Murphy TC**, **Gould JR**, Van Driesche RG, Elkinton JS. Interactions between woodpecker attack and parasitism by introduced parasitoids of the emerald ash borer. *Biological Control.* 2018;122:109-17.
- Tofangsazi N, Morales-Rodriguez A, Daugherty MP, **Simmons GS**, Grafton-Cardwell EE. Residual toxicity of selected organic insecticides to *Diaphorina citri* (Hemiptera: Liviidae) and non-target effects on *Tamarixia radiata* (Hymenoptera: Eulophidae) in California. *Crop Protection.* 2018;108:62-70.
- Yang A, Seabright K, Juzwik J, **Myers S**, Taylor A. Survival of the oak wilt fungus in logs fumigated with sulfuryl fluoride and methyl bromide. *Forest Products Journal.* 2018.
- Chen Z, White MS, **Mack R**, Qiu Z. Steam and Vacuum Treatment of Large Timber in Solid Wood Skids. *Bio Resources.* 2018;13(4):9132-42.





**United States Department of Agriculture**

**Otis Laboratory  
1398 W. Truck Rd.  
Buzzards Bay, MA 02542  
508-563-0900**