United States Department of Agriculture

Otis Laboratory
2019 Annual Report

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

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

It was designed and formatted by: Nevada Trepanowski & Kendra Vieira for Otis Laboratory

Cover designed by: Nevada Trepanowski

OTIS

LABORATORY

ACCOMPLISHMENTS 2019

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Behavioral and Chemical Ecology

Biological Control

Commodity Treatment

Insecticide Technology

Pest Management

Rearing

Survey, Detection, and Analysis

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Molecular Biology

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*Indicates a report describing 19 Riker mount displays created for a PPQ outreach lending collection.

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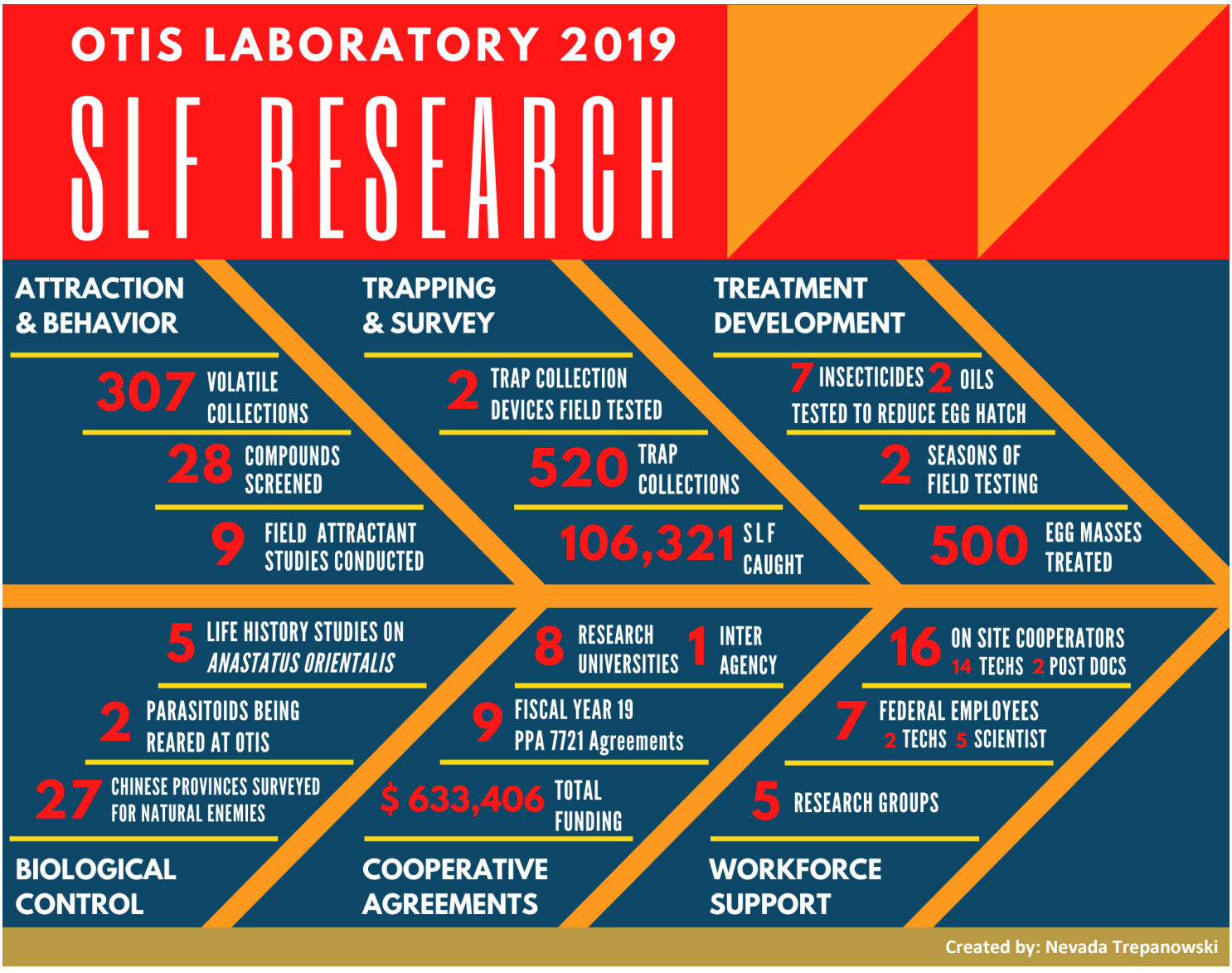
*Indicates a report describing 19 Riker mount displays created for a PPQ outreach lending collection.

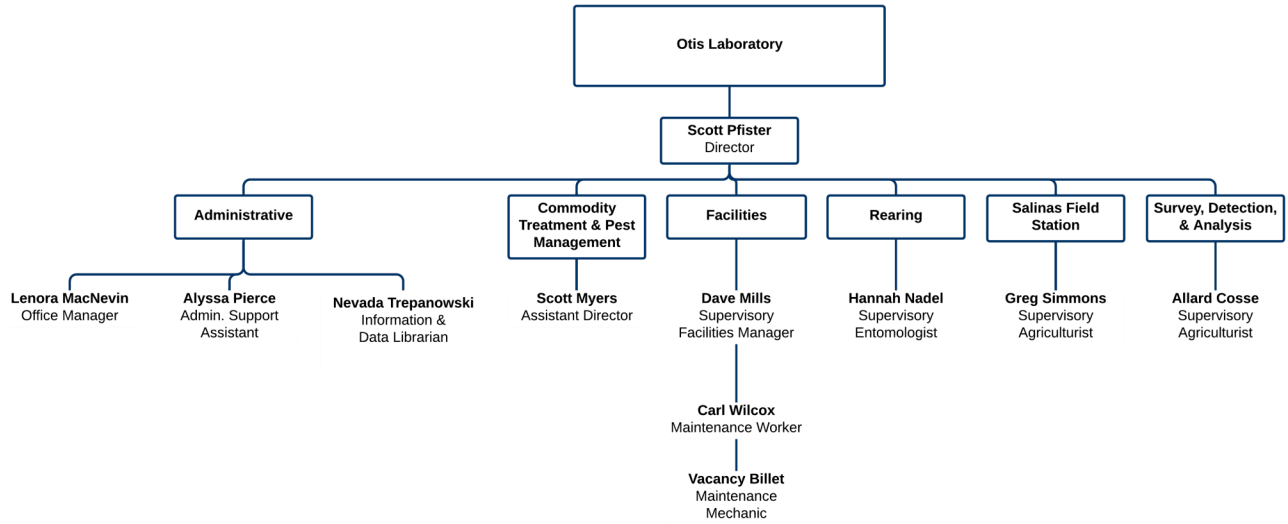
Director's Message

Scott Pfister

Otis has a proven track record of applying interdisciplinary research to support PPQ's pest programs. Our research teams have contributed methodologies that have become critical to the management of gypsy moth, Asian longhorned beetle, emerald ash borer, and many other invasive species. Since 2015 spotted lanternfly has increasingly become the lab's priority pest. Spotted lanternfly research conducted at Otis falls into four broad categories; 1) attraction and behavior, 2) trapping and survey, 3) treatment development, and 4) biological control. The multidisciplinary nature of the SLF work conducted at Otis allows our teams to leverage discoveries made in one area to benefit and influence research in another area; this approach ensures the most dynamic and responsive recommendations are made to the SLF program.

The 2019 Otis Annual Report details the advances that have been made towards understanding SLF and subsequently developing an evidence-based management strategy. Within this report, you will learn about the latest developments in identifying the semiochemicals associated with SLF behavior. You can delve into the research being done to understand the life history and rearing of an SLF egg parasitoid, *Anastatus orientalis*. Additionally, information on the development of new insecticide treatments and trapping tools that have been transferred to the SLF program are also presented. Our researchers' commitment to protecting our nation's forest and agriculture from SLF is apparent by the ingenuity and effort captured in their reports. The metrics depicted in 2019 Otis infographic focuses on highlighting the sheer scale and scope of the SLF research being done at Otis Laboratory.

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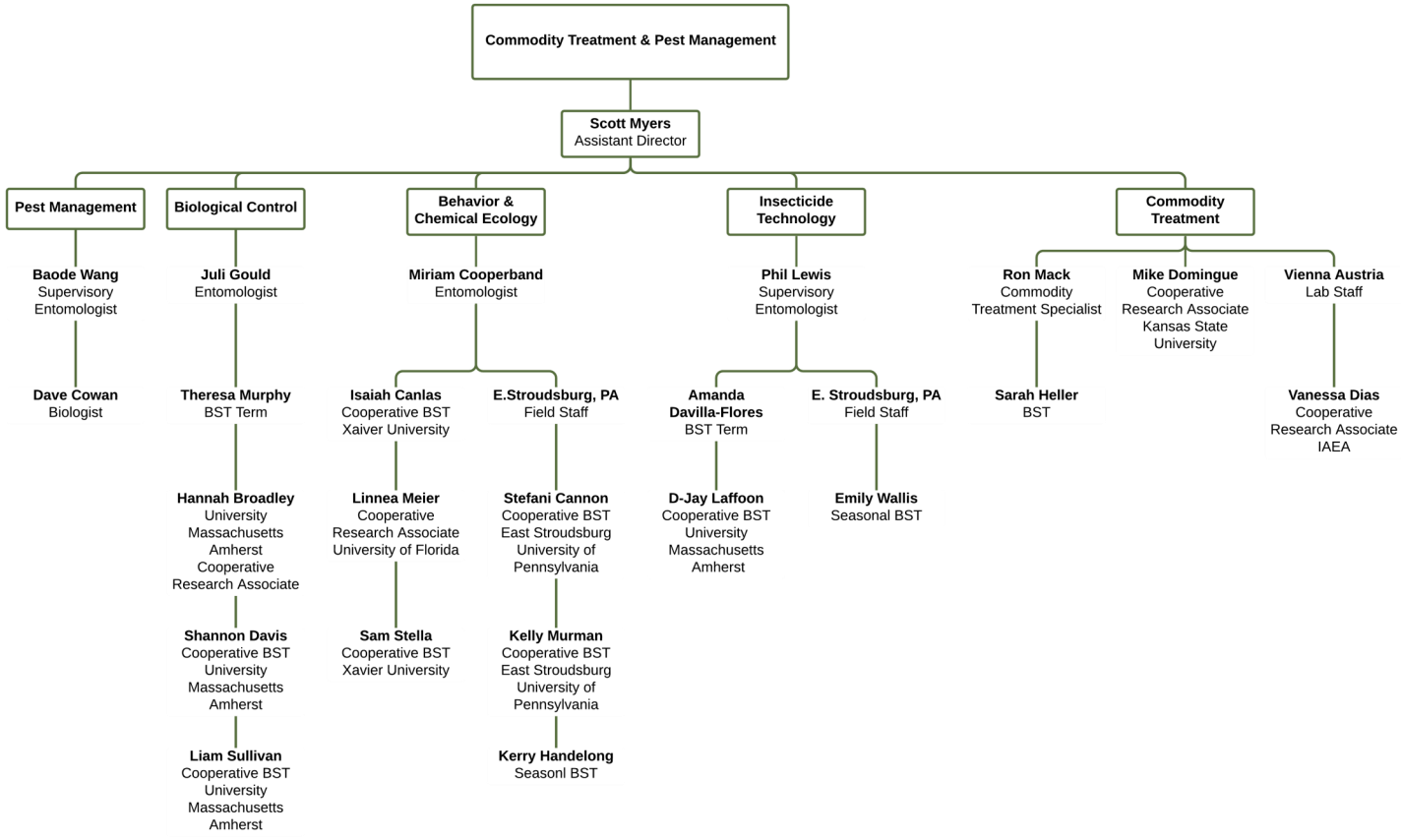
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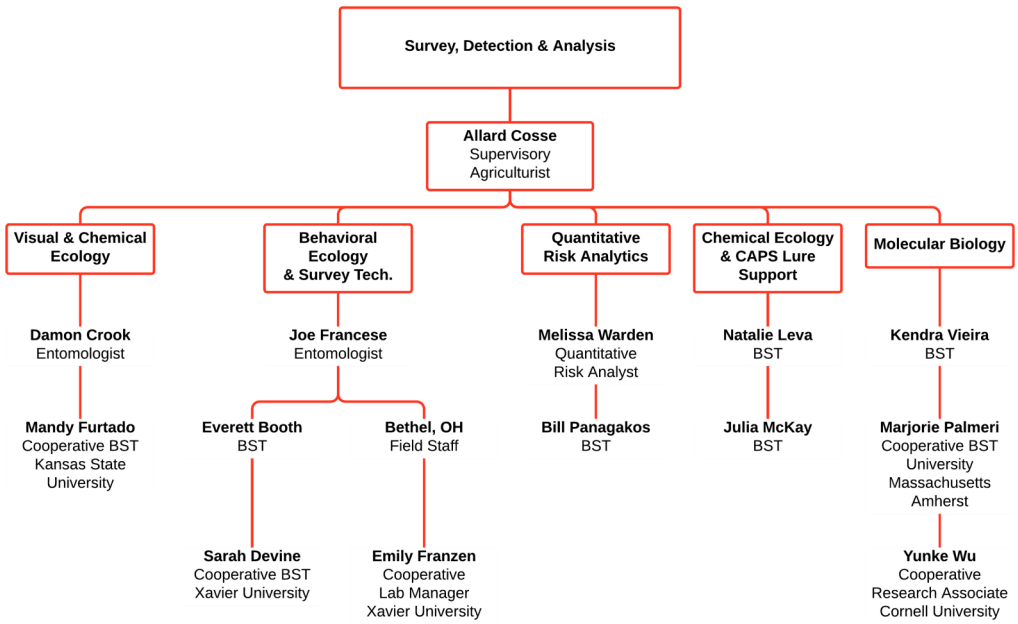
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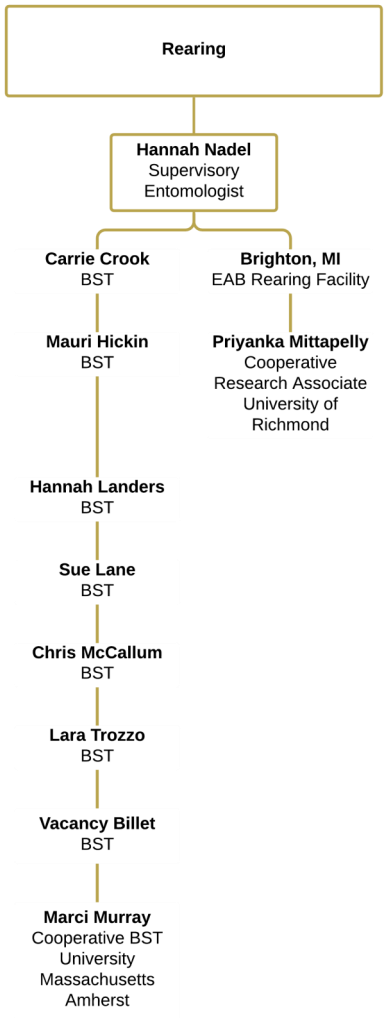
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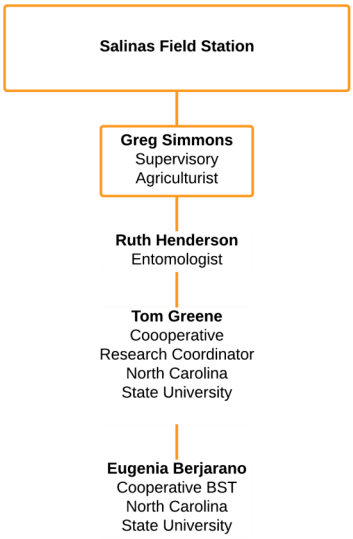
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Semiochemicals and behavior of spotted lanternfly

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Introduction

Spotted lanternfly, SLF, *Lycorma delicatula*, is a polyphagous, phloem-feeding invasive fulgorid native to China. It was found in Pennsylvania in 2014 and has now spread to several neighboring states. This damaging pest feeds on a broad range of over 100 plants but its preferred host is tree-of-heaven, *Ailanthus altissima*, an invasive from China, which is broadly distributed throughout the U.S. Spotted lanternfly can seriously damage or even kill grapevines and threatens a range of other commodities such as fruit trees, nursery stock, and timber [1]. Our previous fieldwork found that, in a low SLF density area, trees "baited" with groups of adult SLF attracted significantly more marked-released SLF than control trees, suggesting SLF use semiochemicals to aggregate [2]. We also found that SLF populations congregate on tree-of-heaven during the two weeks before mating [3]. Our laboratory studies revealed that volatiles from female bodies are attractive to males before mating, suggesting the use of a sex pheromone. We also found that prior to mating, adults form single-sex aggregations on trees. We hypothesized that this unusual behavior could also be chemically mediated. In 2019, we continued investigations to understand the role that chemical communication plays in SLF forming aggregations both as nymphs and adults. We focused primarily on what volatiles are produced and how, and who is attracted to whom. In our search for sex and/or aggregation pheromones, we identified several attractive compounds generated by SLF. Based on laboratory results, we incorporated the most promising compounds into lures for field testing. Additional field tests were aimed at improving our understanding of other SLF behaviors.

Methods and results

In 2019, every week starting from the emergence of first instar nymphs until the beginning of oviposition in October, we collected volatiles in several manners. Volatiles were collected from (1) SLF whole body solvent extracts, (2) headspace of whole SLF bodies, (3) SLF honeydew, and (4) headspace from live SLF on tree-of-heaven; volatiles were also collected from tree-of-heaven without SLF to serve as controls.

The fourth study is still undergoing analysis, however, here we report the findings so far from the first three approaches.

Adults were divided into four stages as previously described [2,4]. A brief description and approximate date ranges of each are as follows:

- Early 1: feeding (August 1-24)
- Early 2: feeding and skewed sex ratios (August 25 - September 13)
- Mid: mating, defined by the first observation of mating in the field (September 14-30)
- Late: oviposition, defined by the first observation of oviposition (October 1 until adult death)

Whole-body extracts

Whole-body solvent extracts contained enormous amounts of cuticular waxes and hydrocarbons relative to volatile components, precluding their injection into the gas chromatograph for electroantennographic detection (GC-EAD) or mass spectrometry (GC-MS). However, these extracts were tested for attraction by all stages of SLF in a dual-choice behavioral bioassay to determine who is attracted to whom, and when. Nymphs were attracted to the whole body extracts of other nymphs, suggesting that an aggregation pheromone may exist for nymphs. We found that males were attracted to extracts from females prior to mating, but not males, suggesting that a sex pheromone may also be involved. Females were not attracted to female extracts, but just after emergence, females were attracted to male extracts (Figure 1).

SLF honeydew volatiles

Honeydew was collected every week and tested for attraction to fourth instars and adults in the olfactometer. Fourth instars were attracted to the volatiles emanating from their own honeydew. Interestingly, adult male and female SLF were only attracted to volatiles from their own honeydew, but not to honeydew from the opposite sex (Figure 1). This discovery may help to explain the skewed sex ratios we have observed and documented in the field every year since we started studying SLF [2, 5, 6]. Although we still do not understand why males and females sometimes aggregate in same-sex groups, this may explain how they find each other to form these aggregations.

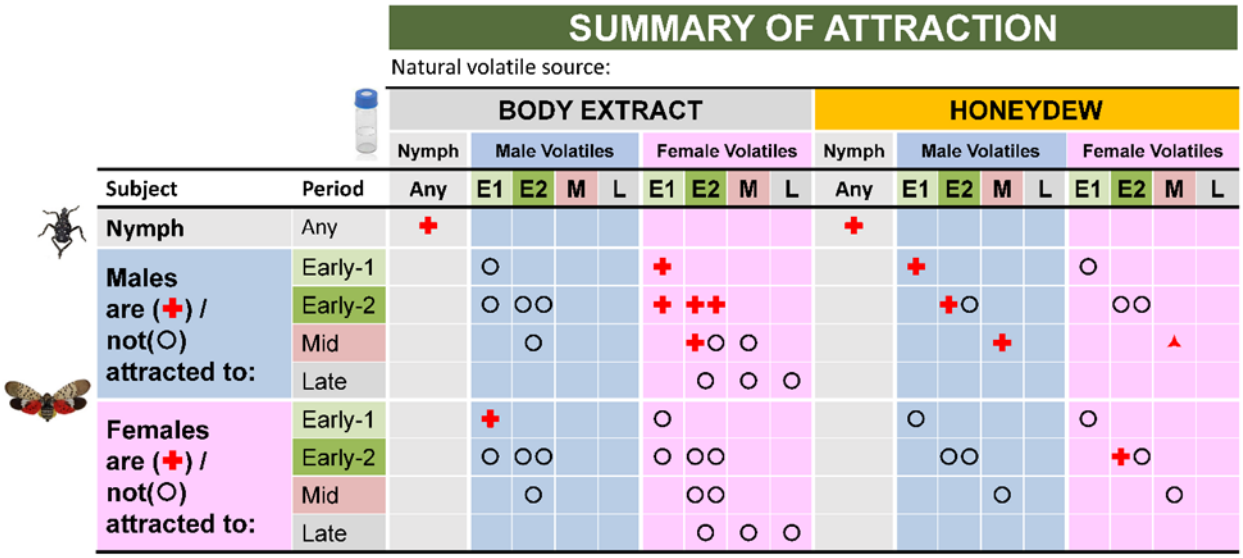

Figure 1. A summary of results from 2018 and 2019 combined, comparing choices between a volatile source and a blank control. Each mark indicates a bioassay series in which the insect (defined on the left) is tested for attraction to natural volatiles from a source defined along the top (nymph or adult Early-1, Early-2, Mid, and Late, as defined in the text). Each ✚ represents a test series resulting in significant attraction to volatiles versus blank controls using a Chi-Square Goodness-of-Fit test ($p < 0.05$), whereas each ○ represents no significant difference. In cases with low n (< 15), ▲ notes a strong trend ($p < 0.1$).

Although the abundance of volatile collections was too low to elicit antennal responses in GC-EAD, 21 compounds were identified using GC-MS. The synthetic versions of these compounds were purchased and screened using GC-EAD, and 10 were found to be antennally active. In addition, six of these compounds were tested and found to be attractive in the dual-choice olfactometer. Two of those compounds, methyl salicylate and sulcatone, were previously identified as kairomones [7,8]. Also, only males were attracted to 2-heptanone, and only females were attracted to 2-octanone (Figure 2).

Whole-body headspace volatiles

Whole-body headspace volatiles were collected weekly from nymphs and adults for use in GC-EAD and GC-MS analysis. Adult males and females were collected separately, and during the two weeks before mating, we analyzed volatiles from both paired and unpaired males and females. Although volatile abundance was not enough to elicit EAD responses, we identified 15 compounds using GC-MS that were purchased or synthesized and screened for an antennal response using GC-EAD. Nine of those were found to be antennally active. Eight were tested for attraction in the dual choice olfactometer, and seven of those were found to be attractive. Two of those compounds, methyl salicylate and sulcatone, were previously identified as kairomones [7, 8]. Interestingly, undecane was attractive to both sexes but did not produce an antennal response in either.

Field testing of attractants

Based on our findings, point source lures were formulated and attached to trees in N blocks using BugBarrier inverted sticky band traps for comparison of different treatments and controls. Field tests to evaluate attraction consisted of the following nine studies:

1. Three dose-response studies spanning 1st, 2nd, 3rd, and 4th instars, comparing 2-nonanone at nine release rates over three orders of magnitude (n = 14, 22, 11)
2. Damaged vs. undamaged *Ailanthus* trees at every stage from 1st instar to late adults (n = 12)
3. Blends of body volatiles against 4th instars to Early-1 adults (n = 12)
4. Blends of honeydew volatiles against Early-1 and Early-2 adults (n = 13)
5. Blends of honeydew volatiles and body volatiles combined against Early-2 and Mid adults (n = 16)
6. Tree damage volatile, ß-ylangene lures, against Early-2 and Mid adults (n = 15)
7. Blends of volatiles preferred by males or females against Mid and Late adults (n = 16)
8. Blends of body and honeydew volatiles combined with kairomones against Late adults (n = 24)
9. Oviposition traps containing blends of body and honeydew volatiles combined with kairomones against Late adults (n = 20)

SYNTHETIC COMPOUND		SOURCE(S) FOUND			SUMMARY OF ATTRACTION									GC-EAD	
					Stage:	Nymphs	Males				Females				
		Live SLF on ToH	Honeydew	Body	Period:	ALL	Early-1	Early-2	Mid	Late	Early-1	Early-2	Mid	Late	Male	Female
Ketones	sulcatone	✓	✓	✓		✚✚			▲	○			▲	○	✚	✚
	2-heptanone		✓	✓				✚				○			✚	✚
	2-octanone		✓	✓				○				✚			✚	✚
	3-octanone		-	✓											✚	✚
	2-nonanone	✓	✓	✓		✚		○	○	✚		✚	○	○	✚	✚
	2-undecanone		✓	-											✚	✚
	2-tridecanone		-	✓											○	○
	Z-6-undecen-2-one		-	✓		○		○				○			✚	✚
Esters	benzyl acetate		✓	-		✚		✚				✚			✚	✚
	methyl salicylate	✓	✓	✓		○✚✚			○			✚✚			✚	✚
	Z-3-nonyl-acetate		✓	-											✚	✚
	nonyl acetate		✓	-											○	○
	isaomyl acetate		✓	-											○	○
	n-decyl acetate		✓	-											○	○
	2-ethyl-hexyl acetate		✓	-											○	○
	ethyl-4-ethoxy benzoate		✓	-											○	○
Hydrocarbons	1-undecene	✓	-	✓		✚									○	○
	undecane	✓	-	✓		○	✚		○		✚		○		○	○
	dodecane	✓	-	✓											○	○
	tridecane	✓	✓	✓											○	○
Aldehyde	decanal	✓	-	✓											○	○
Alcohols	1-octanol		✓	-											✚	✚
	1-nonanol		✓	-											✚	✚
	1-dodecanol	✓	✓	-											○	○
	1-tetradecanol		✓	-											○	○
	1-hexadecanol		✓	-											○	○
	2-phenyl ethanol		✓	✓											○	○
	benzyl alcohol		✓	✓											○	○

Figure 2. A summary of results from 2018 and 2019 combined, showing natural sources where compounds were found (✓), not found (-), or if analysis is still in progress (blank). Synthetic compounds were screened for attraction compared to a blank control in dual-choice olfactometer bioassays, and the results of each insect and stage that was tested are indicated. Each ✚ represents a series of bioassays resulting in significant attraction ($p < 0.05$) to test volatiles over blank controls using a Chi-Square Goodness-of-Fit test, whereas each ○ represents no significant difference. In cases with low N, ▲ notes a strong trend ($p < 0.1$). Finally, antennal activity (✚) or lack thereof (○) was evaluated using synthetic compounds in GC-EAD.

Unfortunately, only one of the above field studies (study #8) resulted in significant differences between treatments, a blend of body, honeydew, and plant volatiles compared to methyl salicylate lures and controls. In that study, methyl salicylate lures caught significantly more SLF than controls, with the blend lure being intermediate. The general lack of significant differences may be due to the high variability in field trials in combination with the relatively low N. Notably, the study with the greatest number of replicates was the only study with significant differences. Future field studies are planned to have a higher number of replicates.

Sex ratio distribution

In previous years we documented highly skewed sex ratios of SLF in the field [5]. In 2019, we hypothesized that when males disappear from trunks before the first observation of mating, they may be heading to the canopy to court females, and that females that are not actively seeking to mate prefer to feed on the trunk, thus causing the documented changes in sex ratio. To test this hypothesis, a mark-release-recapture study was conducted using dinotefuran treated *Ailanthus* trap trees with both BugBarrier sticky bands to capture SLF on the trunks and tarps beneath trees to capture SLF that died after feeding higher up in the trees (N = 5). If our hypothesis is correct, we would expect to see different sex ratios on trunks (BugBarrier) than in the canopy (tarps) at different times. Each week for seven weeks, equal numbers of male and female SLF were captured, marked and released on each of the trap trees, and traps and tarps were tallied after 24 hours and one week. The null hypothesis was that SLF that were released at a 50:50 ratio would be recaptured at a 50:50 ratio unless there was a specific draw for one sex over another to either the trunks or canopy, which would result in a sex ratio bias of marked-recaptured individuals on either or both the BugBarrier or tarps. The total recapture rate was 16% over seven weeks. We found that the natural population (unmarked SLF) was significantly male-biased on both BugBarrier and tarps until the week mating was first observed (September 9), at which time the sex ratio was 50:50 for a week. After that, the sex ratio on both BugBarrier and tarps became significantly female-biased (Figure 3).

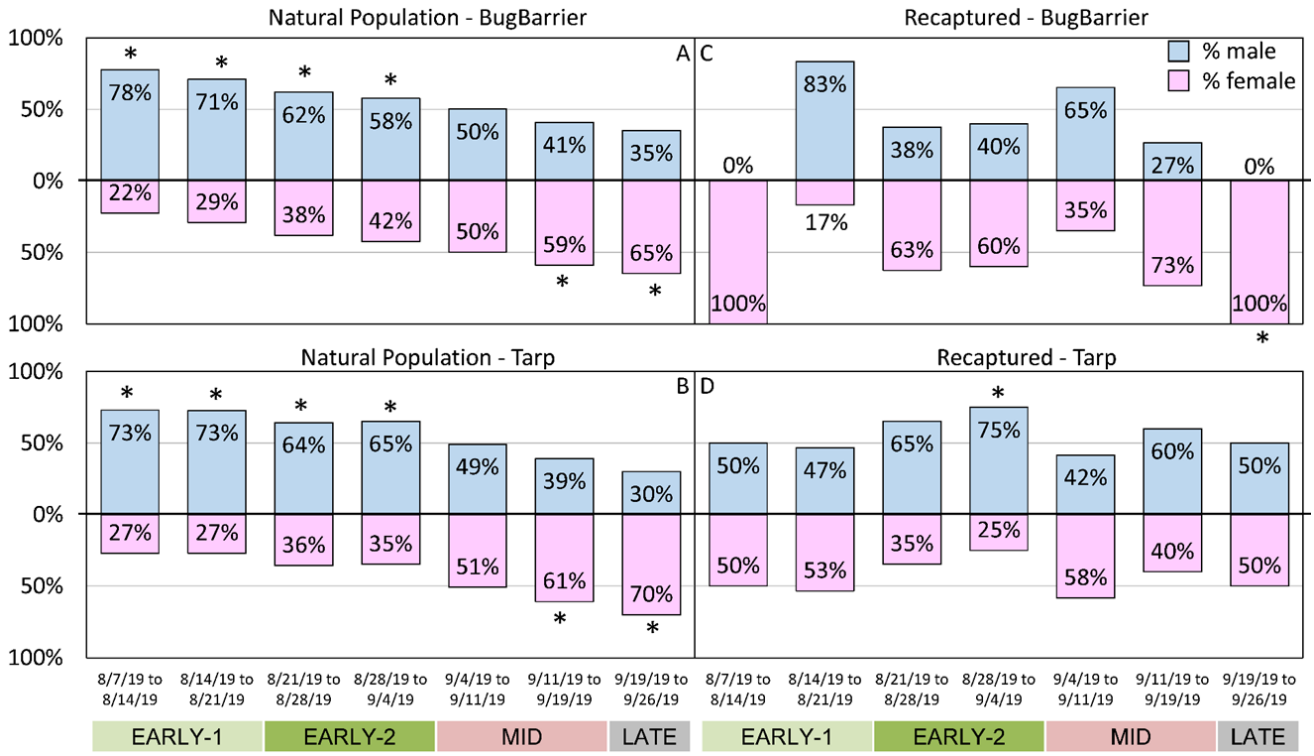

Figure 3. Sex ratio of the naturally occurring SLF population (A, B) and SLF that were marked and released at a 50:50 sex ratio then recaptured (C, D) on BugBarrier on trunks (A, C), and tarps (B, D) beneath the canopy of trap trees (N = 5) over seven weeks. Asterisks indicate when the sex ratio was significantly different from 50:50 (Chi-Square Goodness of Fit test, $\alpha = 0.05$).

Although the number of marked-recaptured SLF was low, there were two times when the sex ratio of recaptures was significantly biased. During the week of mating, the sex ratio of recaptured SLF on tarps was predominantly male, suggesting males at that time headed to the canopy to feed. Additionally, at the end of the Mid stage (mating period), the sex ratio of recaptured SLF on BugBarrier was predominantly female, suggesting that females preferred to spend time on the trunks at that stage. However, we did not observe the expected sex ratio differences between trunk and tarp in the natural (unmarked) population. The strongly biased sex ratios that change over time suggest that males and females aggregate in different places at different times. Locations of females during Early-1 and males during Mid is still poorly understood. It may be explained by male aggregations occurring on some trees while female aggregations occur on other trees, which is what we observed in previous years. In this experiment, the five trees had similar sex ratios.

Tracking SLF in the field

In 2019 we experimented with two types of tracking technology to follow the movement of adult SLF in the field. Techniques for using radio telemetry (RT) and harmonic radar (HR) to track SLF movement from a distance were developed (Figure 4).

Figure 4. Early attempts at attaching a harmonic radar tag (left) and a radio telemetry nanotag (right) to the pronotum of adult SLF. Subsequent testing revealed that a better attachment point for the nanotag was a ventral abdominal attachment which interfered less with flight.

In HR, the receiver sends a signal, which is reflected by a passive tag that is attached to the insect, and the receiver detects the reflected signal. The advantage of this technology is that the tags are lightweight so they are less likely to impede movement, and they don't require a battery so they can be detected indefinitely. The disadvantage of HR is that it cannot detect the tag from more than 10-50 m away, increasing the chances that long-distance travelers will be lost. In addition, unless you can see the insect and it is marked, there is no way of knowing which insect you detect, and this is problematic when using more than one start date or location. RT uses a "nanotag" transmitter that contains a battery and is attached to the insect. Its advantage is that it can be detected as far as 500 m away, and each tag produces a uniquely identifiable signal so you can keep track of the movement of many insects starting from different days and locations. The disadvantages of the nanotag are that it is heavier, which may impede the insect's movement, and the battery has a limited life of about three weeks. We developed and tested techniques using both technologies. The RT nanotag provided the best results, with maximum recovery of a living female 434 m away from the release point after 10 days (Table 1, Figure 5).

One living female was found 197 m away from the release point two days after release, which represents the fastest rate of movement we were able to calculate. We were the first to test a new nanotag, the smallest available weighing only 150 mg. SLF were able to carry them and even fly with them. Recovery of insects was less efficient using harmonic radar (Table 1), and as such, future work should focus on using radio telemetry to quantify the extent of movement by adult SLF. Unfortunately, nymphs are too small to carry nanotags, so harmonic radar is still the best option to track nymphs.

Table 1. A comparison of radio telemetry and harmonic radar systems used to track adult SLF in the field, and summary of data.

	Radio Telemetry	Harmonic Radar
Total SLF Released	97	43
No. Recovered 3+ m (% of total)	35 (36%)	5 (12%)
No. Recovered < 3 m (% of total)	29 (30%)	1 (2%)
No. Recovered on Release Tree (% of total)	16 (16%)	9 (21%)
Total No. Recovered (% of total)	83 (86%)	22 (51%)
Max. Days Recovered	20	31
Females		
Avg. Distance Moved (m)	29.8	8.5
Max. Distance Moved (m)	434.0	60.4
Max. Rate (m/d)	52.1	3.4
N	41	8
Males		
Avg. Distance Moved (m)	3.1	4.8
Max. Distance Moved (m)	14.9	25.3
Max. rate (m/d)	7.5	2.5
N	40	7

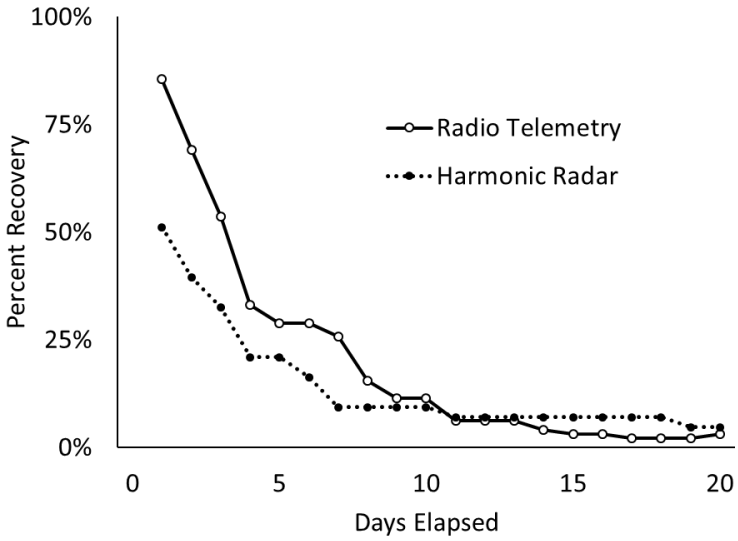

Figure 5. Recovery rates of radio telemetry and harmonic radar when tracking SLF in the field.

Conclusions

We have now documented that SLF use semiochemicals, found both in body volatiles and honeydew, to find each other. Nymphs were attracted to the volatiles from honeydew and bodies of other nymphs, suggesting a possible aggregation pheromone. Before mating, males were significantly attracted to female body extracts, but not to male body extracts, which suggests the presence of a sex pheromone. Conversely, males were significantly attracted to honeydew produced by other males, but not by females, which may provide a mechanism for the strongly biased sex ratios seen in the field. Of the identified compounds, eight compounds from the two sources were found to be attractive, some of which were attractive to only one sex or the other. Other compounds still need to be tested. When tested in the field as point source lures, no significant differences were seen. This may be a problem with the odor delivery in the field, as a point source is not the form of volatiles being emitted from large aggregations of SLF and honeydew coating large areas. Future work will focus on determining exactly which compounds are important in aggregation and mate-finding, collecting larger amounts of volatiles for use in GC-EAD to identify any trace compounds that may be important in attraction, and development and testing of novel lure deployment systems that seek to mimic natural sources of semiochemicals such as honeydew, which are not encountered as a point source in nature.

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Optimizing release of parasitoids against the emerald ash borer

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Release of parasitoids against emerald ash borer, EAB, *Agrilus planipennis*, began in 2007 in the Midwest and has expanded to 25 states as populations of EAB continue to spread throughout the U.S. State and university stakeholders are critical partners in the release and recovery of EAB parasitoids. Recovery data are used by researchers at Otis to determine where parasitoids are successfully establishing. In the early years of EAB biocontrol, two larval parasitoids (*Tetrastichus planipennisi* and *Spathius agrili*) were released in northern states. *Tetrastichus planipennisi* established well and spread out from the release sites. However, while *S. agrili* could overwinter successfully, populations of *S. agrili* did not persist past a year or two. Climate matching analysis suggested that *S. agrili* might be better suited to the southern U.S. To identify a *Spathius* species that might be a better climatic match for release in the northern U.S., explorations were conducted in Russia, where *S. galinae* was ultimately collected.

Research on the life-cycle of both EAB and the three larval parasitoids showed that both *T. planipennisi* and *S. galinae* emerge early in the spring. For the parasitoid populations to persist, emerging adults must encounter mature EAB larvae for oviposition. Therefore, *T. planipennisi* and *S. galinae* are best suited for areas where at least some EAB exhibit a two-year life-cycle. *Spathius agrili*, on the other hand, emerges over a month and a half after adult EAB emergence and is compatible with EAB populations that undergo a one-year life-cycle. Generally, EAB in the north are more likely to experience a two-year life-cycle than those in the south, but the proportion of EAB overwintering as larvae (and thus available for parasitization in the spring) throughout the U.S. was previously unknown. The study detailed here was initiated to determine the proportion of EAB overwintering as larvae throughout the country and to model the probability of parasitoid establishment.

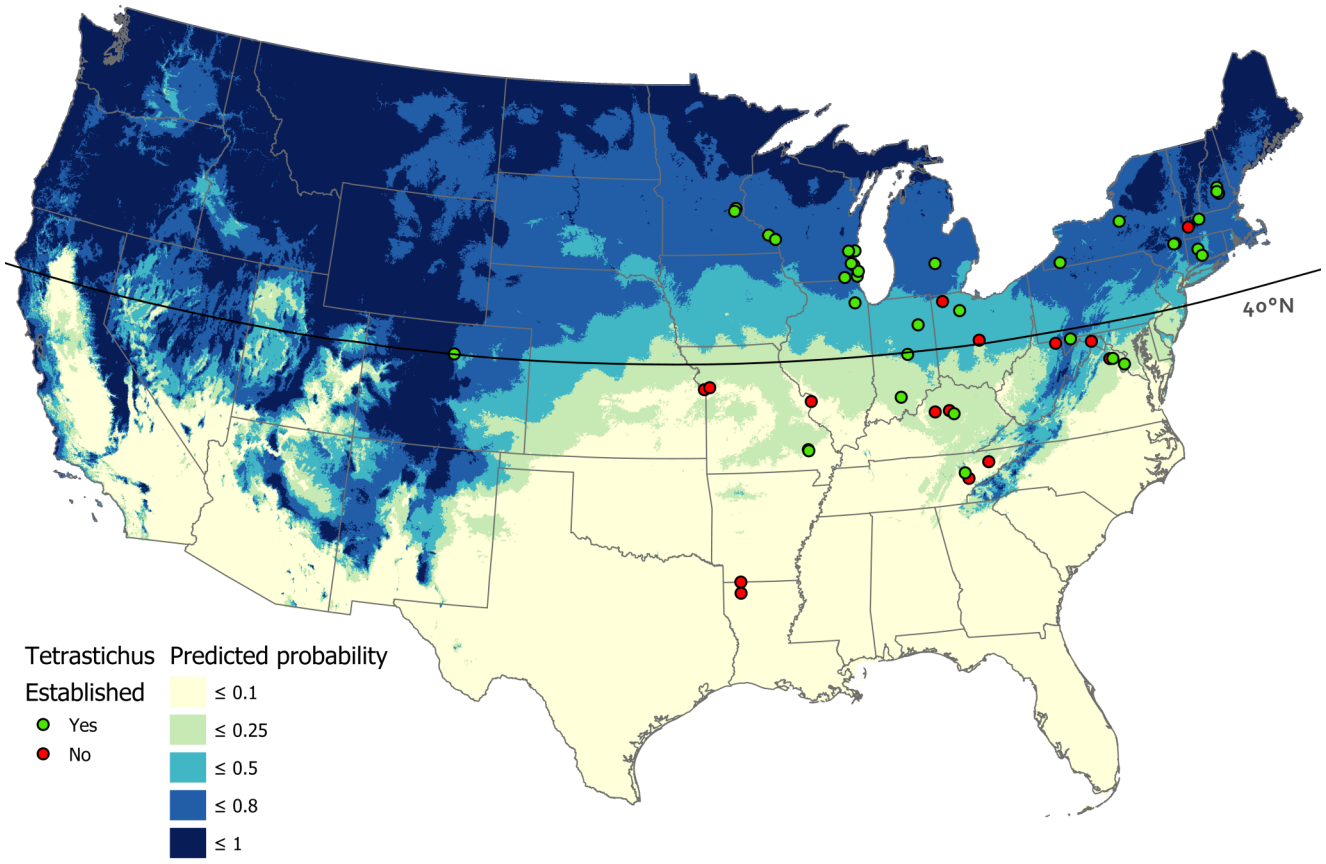

Figure 1. The estimated proportion of EAB that overwinters as larvae (not J-larvae) throughout the U.S. based on growing degree days (base 50°F) accumulated from January 1 to September 30 the previous summer. Green dots indicate locations where *T. planipennisi* established, and red dots indicate locations where samples were collected, but the parasitoid was not found.

Collaborators felled ash trees at over 90 sites in 22 states and recorded the proportion of EAB that overwintered as larvae (and were available for parasitization) or J-larvae in overwintering chambers (and were not available for parasitization). We found that the number of growing degree days (GDD) base 50°F that accumulated the previous year between January 1 and September 30 was a good predictor of the proportion of EAB that overwinters as larvae. This relationship allowed us to model the proportion of EAB overwintering as larvae based on summer temperatures (Figure 1). The establishment of *T. planipennisi* was also correlated with the percentage of those overwintering as larvae. As predicted, the likelihood of establishment was higher as the proportion of EAB available as larvae in the spring increased (Table 1). The threshold for expected establishment of *T. planipennisi* occurred at 13% of EAB overwintering as larvae (Figure 2), which indicates that *T. planipennisi* can rebound from the bottleneck of larval availability in the spring. This species is very good at finding its host, has a high rate of reproduction, and has multiple generations per year. As our planet warms, our model predicts that parts of the southern U.S. will become increasingly unsuitable for the persistence of *T. planipennisi*, especially in the center of the country (Figure 2).

Table 1. Relationship between the percentage of EAB overwintering as larvae and the probability of establishment of *T. planipennisi*.

Percentage of EAB overwintering as larvae	Likelihood of *T. planipennisi* establishing	Growing degree day threshold
51-80%	92%	2,985
26-50%	78%	3,500
11-25%	50%	3,975
0-10%	23%	-

Because *S. galinae* also emerges early in the spring and requires EAB overwintering as larvae, there is a chance that our model may apply to this species as well. Efforts are underway to release *S. agrili* in the south, but to date, we do not have data on the establishment of this species.

Results from this study detail, for the first time, a comprehensive map of expected locations for one-year versus two-year EAB life-cycles across the U.S. and, furthermore, where *T. planipennisi* establishment is expected to occur. This information is a useful tool for the EAB biological control rearing facility as well as state, university, and federal stakeholders in identifying which parasitoid species will likely establish at a particular location, allowing for the efficient and effective allocation of biological control resources.

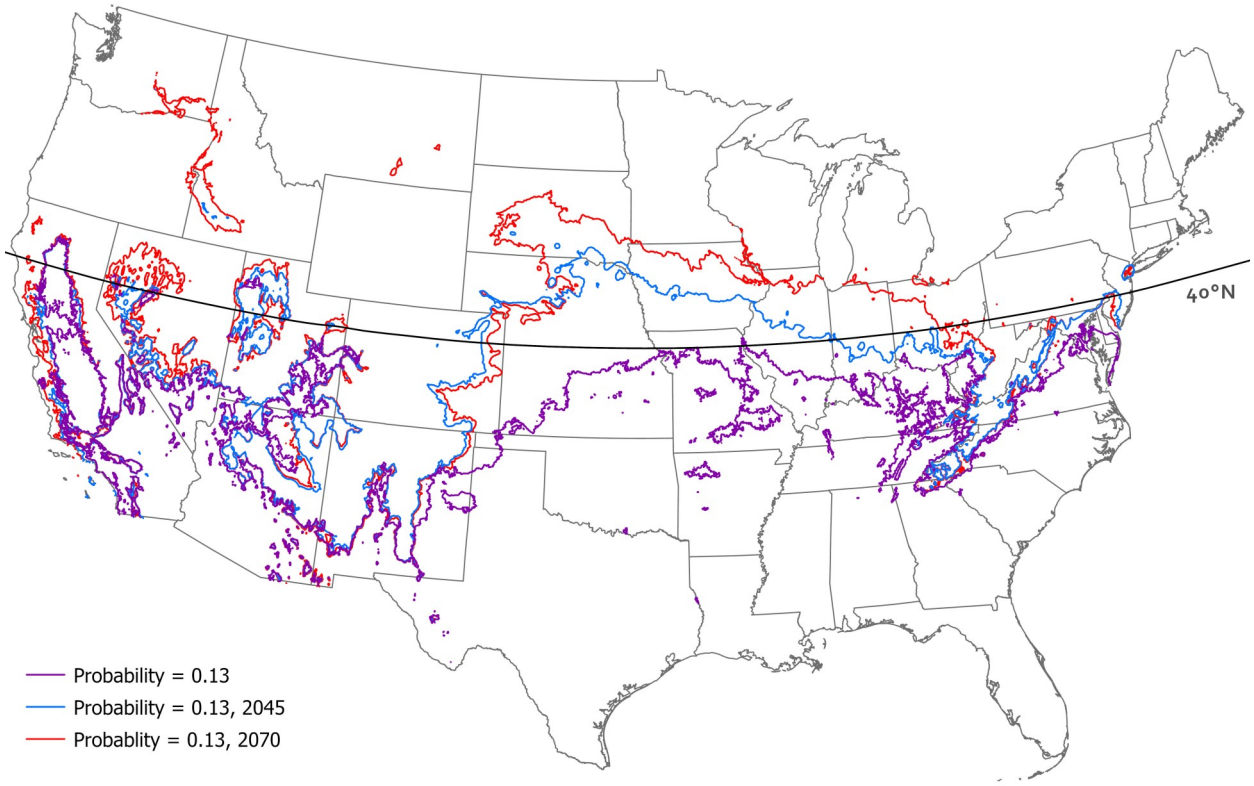

Figure 2. Thresholds for predicted establishment of *T. planipennisi* and how areas suitable for *T. planipennisi* establishment are expected to change with the projected increase in summer temperatures.

Life history and rearing of *Anastatus orientalis*, an egg parasitoid of the spotted lanternfly

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Introduction

To support the goals of the Spotted Lanternfly Program, research is being conducted to develop classical biocontrol methods for spotted lanternfly, SLF, *Lycorma delicatula*. To date, two potential biocontrol agents from China have been identified: an egg parasitoid *Anastatus orientalis* and a nymphal parasitoid *Dryinus sinicus*. *Anastatus orientalis* is of particular interest because a parasitoid from this genus, likely *A. orientalis*, was found parasitizing SLF egg masses in South Korea, where SLF also is invasive [1,2]. The research detailed here focuses on investigating the biology (longevity, fecundity, life-cycle, etc.) and rearing of *A. orientalis* to asses its potential efficacy in a biocontrol program and to provide the information necessary for developing a rearing protocol.

Specimen collection

Laboratory colonies of *A. orientalis* were established from parasitized egg masses collected from the field in Beijing, China (over 150 parasitized egg masses collected between 2016 and 2019). The *L. delicatula* egg masses used to maintain the wasp colony and for these studies were field-collected from Lyon Station, Pennsylvania in April 2018 and from Lansdale, Royersford, and Leesport, Pennsylvania in March 2019.

Adult longevity and fecundity

To estimate longevity and fecundity of adult *A. orientalis*, a single newly emerged (<24 hours old) male and a single female were placed together with one SLF egg mass in a medium-sized rearing container (16-oz plastic deli cup) under conditions set to mimic Beijing in the early fall (daily high of 25°C and low of 14°C, 12.5: 11.5 L:D, 65% RH). Hereafter, these conditions will be referred to as Beijing fall conditions. One SLF egg mass was given to each pair twice per week until the female died. After one month in Beijing fall conditions, the egg masses were moved to 25°C, 16 light:dark, 65% RH (hereafter, referred to as 25°C/long-day conditions). Once progeny began to emerge, adult wasps were removed every other day and the date of emergence and the sex of each emerging wasp were recorded. The egg masses from the first round were dissected to determine the fate of any un-emerged eggs. Thirty replicates were run.

Female wasps lived significantly ($t = 9.0105$, $df = 29$, $p = <0.0001$) longer (68.2±4.1 days) than the male wasps (23.0 ± 1.9 days). On average, females produced 94.3 ± 8.6 (mean ± SE) total offspring that successfully emerged as adults. Females produced most of their progeny between weeks one and four (Figure 1).

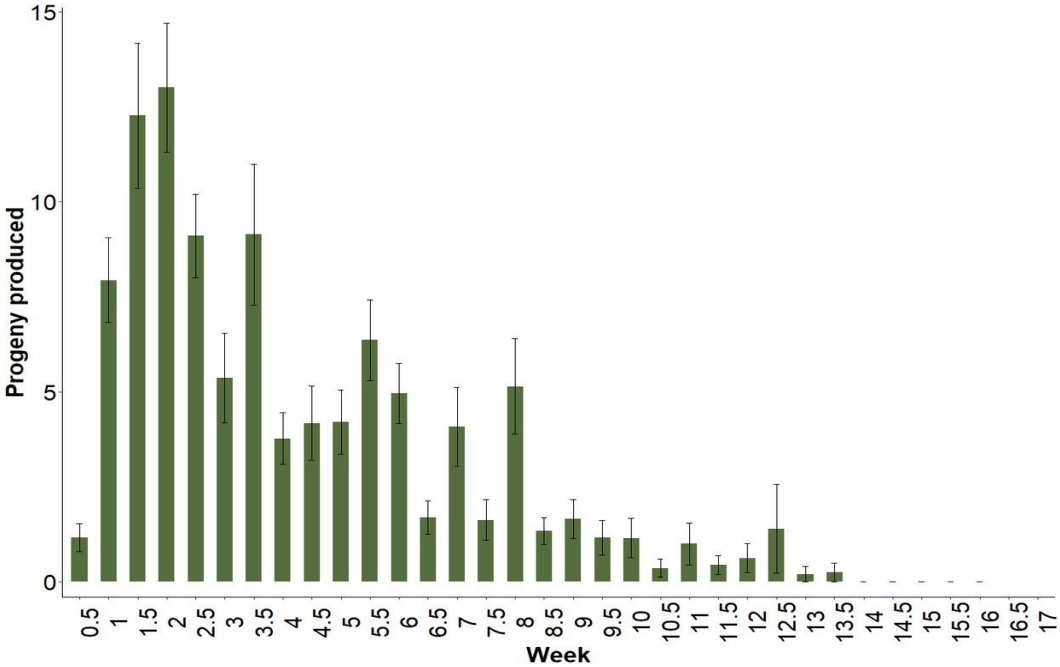

Figure 1. The average number of progeny produced by *Anastatus orientalis* females over their lives. The bars show means and standard errors.

Evidence of a pre-oviposition period was noted, with only eight of the 30 females producing any progeny in the first half week. Considering only females that laid eggs, only 3.6 progeny were produced, on average. The sex ratio of the progeny was highly female-biased at first and became progressively more male-biased; by week 13 only male progeny were produced (Figure 2).

Impact on *A. orientalis* host feeding on SLF mortality

The impact of *A. orientalis* host feeding on SLF mortality was investigated. Newly emerged (< 24 hrs old) female and one newly emerged male *A. orientalis* were placed in a medium-sized rearing container with honey and an egg mass for one week. Simultaneously, a set of control egg masses were run with the same conditions but with no exposure to wasps. After exposure, the egg masses were allowed to develop for one month before being moved to 25°C conditions). The number and sex of the progeny that were produced from each egg mass were recorded.

There was no evidence of a significant effect of wasp host feeding on the mortality of the SLF eggs (df = 28, pseudo $R^2 = 0.18$, $p = 0.0163$). When egg masses were exposed to wasps, a mean of 14% of the egg masses died due to non-parasitoid causes (i.e., they produced neither a SLF nymph nor an *A. orientalis* wasp), while 46% of the control egg masses that were not exposed to wasps died due to non-parasitoid causes. This trend is opposite to what was expected.

Oviposition and chill requirements for parasitized egg masses

A third experiment was designed to test the production of progeny when oviposition occurred under the two previously described conditions (Beijing fall conditions and 25°C) and with different storage conditions of the parasitized egg masses (storage in chill at 5°C for 0, 1, 2, 4, and 6 months). Oviposition and chill requirement information is necessary for mass rearing and stockpiling for release, should these parasitoids be approved as a biocontrol agent. Five females and one male that had recently emerged (<48 hours old) were placed in a medium-sized rearing container with honey. Half of these replicates (n = 50) were kept in Beijing fall conditions and the other half (n = 50) in 25°C. Each set was given two SLF egg masses for one week. The parasitized egg masses were held for another month under the same conditions to allow for development before being moved to their respective chill conditions (storage in chill at 5°C, full dark, 65% RH for 0, 1, 2, 4, and 6 months). They were then moved to 25°C for emergence. Any wasps that emerged were removed within 48 hours and the number that was produced from each egg mass was recorded. Two weeks after emergence had ceased, the egg masses were dissected to determine the fate of any un-emerged eggs.

There was high progeny emergence when oviposition occurred under Beijing fall conditions, but almost all the progeny went into diapause when oviposition occurred under constant

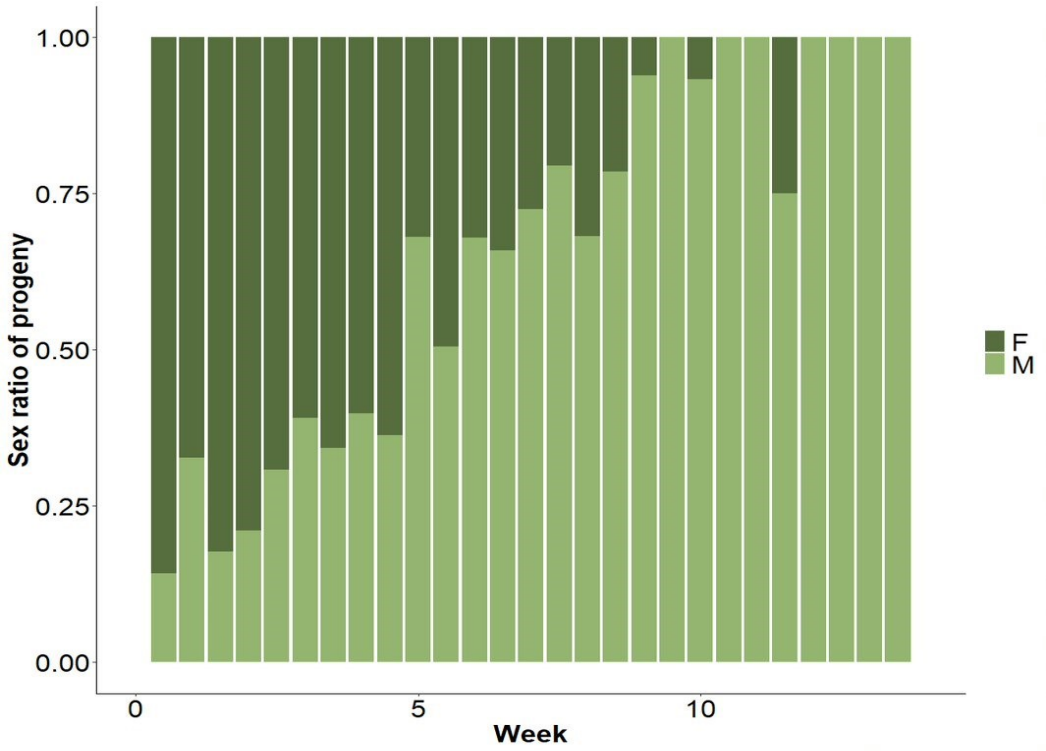

Figure 2. The sex ratio of the progeny produced by *Anastatus orientalis* females over their lives with no re-mating.

25°C/long-day conditions (Figure 3). Further, the longer we held the "Beijing fall" egg masses in chill, the more mortality the progeny wasps experienced. We do not yet know how to break the diapause that the wasps experienced under the 25° C/long-day conditions but future studies are planned.

Since SLF egg masses are only available in the field from December to May, it is important that we are able to utilize both freshly laid egg masses and those that are many months old to conduct rearing year-round. We found no significant difference (p = 0.24) in *A. orientalis*' ability to use newly collected egg masses as compared to stored egg masses. From the new eggs, an average of 12.0 ± 2.6 (mean ± SE) progeny were produced, while 15 ± 2.3 progeny were produced from the stored eggs.

Conclusions

These results show that female *A. orientalis* females are long-lived, but that they produce the most offspring after a one-week pre-oviposition period and before their fourth week. The sex ratio of progeny begins as female-biased, but with no additional mating (most males were dead after 23 days), then shifts to a male-dominated sex ratio. Surprisingly, while we observed host feeding behavior, we did not document additional mortality to the SLF egg masses. This also warrants further investigation. In contrast to Seo et al., we found that we could not produce a continuous colony under constant 25° C temperature/long-day conditions [3]. It may be that the strain of *A. orientalis* used in the studies documented here is different than was studied in Asia. Lastly, we confirmed that stored SLF egg masses were just as efficient in producing wasps as freshly collected egg masses. Together, these findings were used to inform the current rearing protocol used by the USDA APHIS. Specifically, we now include a one-week pre-oviposition period, expose the egg masses to wasps in Beijing fall temperature and light conditions, use young females, provide honey to limit host feeding, use stored SLF egg masses, and hold parasitized egg masses in chill for less than a month.

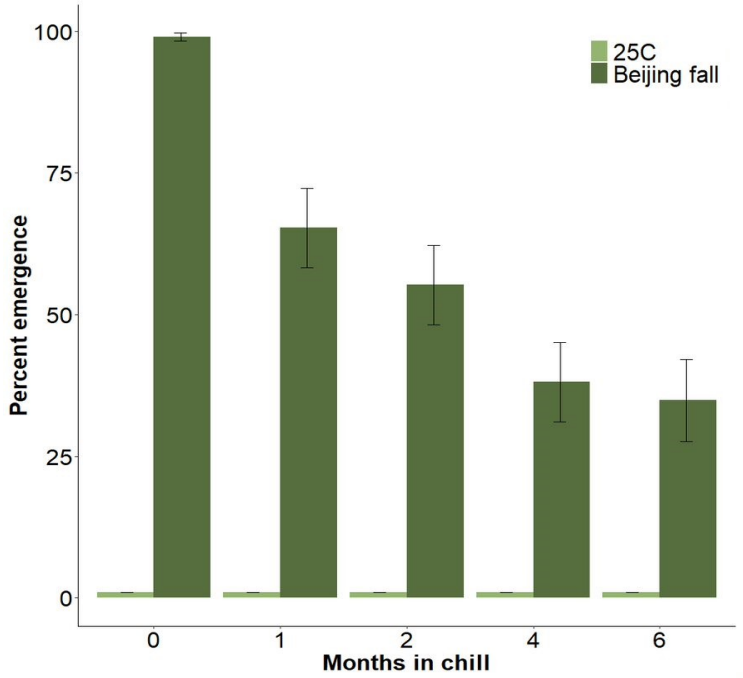

Figure 3. Results from the study on oviposition and chill requirements showing percent emergence of progeny oviposited under 25°C and long-day conditions versus in fluctuating temperatures emulating Beijing fall conditions and held for 0, 1, 2, 4, or 6 weeks in chill (5°C).

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Progress towards Asian longhorned beetle biological control using sentinel logs

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Introduction

Otis's Biological Control Group has been researching the biological control of Asian longhorned beetle, ALB, *Anoplophora glabripennis,* in support of the ALB Program's ongoing eradication goal. Successful eradication efforts have led to declining ALB populations that have become increasingly more difficult to detect. Host-specific natural enemies may aid in locating and killing ALB remaining in the environment. In addition to conducting foreign exploration for ALB natural enemies, we are exploring the possibility of using parasitoids native to the U.S. in an inundative biocontrol program. Our previous work identified a parasitic wasp, *Ontsira mellipes*, as being effective at readily parasitizing ALB. A crucial step in determining the feasibility of an inundative biocontrol approach is to deploy sentinel logs in the field to assess the search efficiency of parasitoids in the field and thus test their efficacy as biological control agents. However, we understand the importance of conducting this research with caution and sensitivity. ALB infested trees are destroyed whenever located, including trees belonging to homeowners as well as street trees. The optics of the USDA putting out ALB infested wood in those same areas is challenging, and we have a responsibility to assure our stakeholders that deploying sentinel logs is a critical step towards the control of ALB and will be carried out with the utmost caution.

The research reported in this report explored the safety of using sentinel logs from multiple angles; we wanted to 1) test the logs themselves to see if ALB adults could survive and emerge from lab-produced sentinel logs, 2) test cages that could hold the logs to ensure that if adults did emerge, they would not be able to escape, and 3) test those same cages with our parasitoids to ensure that caging the logs did not disrupt parasitoid searching and undermine our research goals.

ALB survival in sentinel logs

To investigate if larvae could survive in our sentinel logs, ALB adults were exposed to cut striped maple logs (1 m in length) to allow for infestation. The resulting larvae developed in the Otis Containment Facility from August to December. The logs were then removed from the Otis Insect Containment Facility and placed outside. To prevent tampering and to ensure that the ALB had no chance of escaping, the logs were placed in locked barrels in a secure shipping container.

Before logs were placed outdoors, we peeled a subsample to determine which larval instars would be exposed to overwintering conditions. The logs were brought back inside in May, and additional subsamples were peeled to examine overwintering mortality. An average of 79 ± 19% mortality was found in 2017 and 20 ± 23% mortality in 2018. The logs were kept in barrels in the Otis Insect Containment Facility over the summer and fall to see if any adults would emerge. In November, additional subsamples of the logs were peeled; 100% mortality was observed, prompting the peeling of the remaining logs. This experiment was conducted in 2017 and repeated in 2018, with a total of 97 logs and 1,071 larvae.

ALB has several larval instars, and it is impossible to distinguish the instars through visual examination, especially for dead larvae (Figure 1). Therefore, head capsule size was used as a proxy for instar to interpret our results based on the work of Keena and Moore (2010), which measured the head capsule size of ALB at different instars, [1]. The results are shown in Table 1. Larval feeding location was also used to determine instar as described by Yan, and Qin (1992) who found that 1st instars feed on the rotting cambium, 2nd and 3rd instars feed on healthy phloem and outer layers of xylem, and late 3rd and later instars feed on the xylem [2]. ALB needs to reach at least 6th instar to pupate, although the majority require more instars [1].

Figure 1. Desiccated larvae peeled from sentinel logs in November 2018.

Although head capsule measurements suggested that some of the larvae that survived the winter may have reached the 6th instar, this proportion was incredibly small (Table 1). Additionally, the feeding data do not indicate that any of the larvae matured past an early stage. No larvae survived to the adult stage, and 0% of the 773 larvae recorded in the 75 logs that were kept over the summer moved past feeding on the outer layers of xylem.

Table 1. Results of peeled overwintering logs in 2017 and 2018. A subsample of logs was peeled before overwintering to assess which larval instars were being tested. A subsample of logs peeled in May was used to assess overwintering mortality. The remainder of the logs were peeled after one year and were used to assess the most advanced instar the larvae reached in our sentinel logs.

	Percentage of larvae at each instar									Total # of logs peeled	Total # of larvae found
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th		
2017 logs before OW	14.1	23.4	43.8	15.6	3.1	0	0	0	0	3	64
2017 logs in May	2.8	55.6	38.9	2.8	0	0	0	0	0	5	36
2017 logs remaining	17.5	35.4	31.5	10.7	3.6	0.6	0.3	0	0.3	34	308
2018 logs before OW	65.6	26.4	8.0	0	0	0	0	0	0	7	125
2018 logs in May	34.7	34.7	16.7	13.9	0	0	0	0	0	7	72
2018 logs remaining	51.8	27.6	19.2	1.5	0	0	0	0	0	41	475

Sentinel log containment

Cage construction was explored to develop a chew-proof cage that would prevent ALB adults from escaping after emerging from a sentinel log. Different materials were tested against newly emerged ALB adults to see if they could chew through the material to gain access to food. None of the adults were able to chew through the ¼" steel or ⅛" steel mesh. To test if emerging adults would be able to escape the cages, artificially infested logs with mature ALB larvae were used with two potential cage designs: a wrapped cage and a suspended cage (Figure 2). Emerging adults were not able to chew their way out of either cage design.

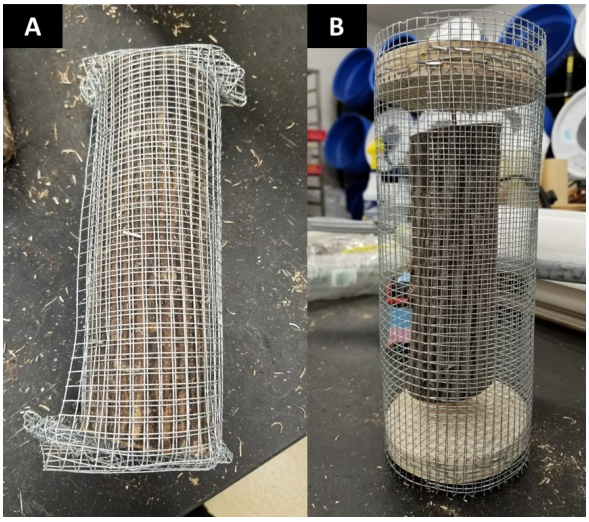

Figure 2. Two different cage designs for sentinel logs were tested: A) a wrapped cage design and B) a suspended cage design.

Finally, we tested whether *Ontsira mellipes* would find and attack ALB inside a steel mesh cage. In the experiment, five females and one male were exposed to two sticks containing ALB larvae. One stick was caged in steel mesh, and the other was uncaged. We found that the steel cage did not discourage oviposition. There was no significant difference in the frequency of attack or the number of offspring produced.

Conclusions

Although our field trial protocol calls for retrieving the sentinel logs one week after deployment, our research shows that even if logs are not retrieved, there is a very low risk of any ALB escaping into the environment. Sentinel logs will not stay suitable long enough to allow ALB larvae to complete development and potentially emerge as adults. In addition, by caging all sentinel logs as an added precaution, even if an adult did manage to emerge, our caging would ensure that an adult ALB would not be able to escape and enter the environment. We are confident that our results demonstrate that sentinel log research can be conducted safely.

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Effect of low-oxygen conditioning on radiotolerance of four tephritid fruit fly species

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Introduction

Phytosanitary irradiation (PI) has been successfully used to disinfest commercial fruit and facilitate international agricultural trade. The use of generic treatments against groups of quarantine insect pests has significantly contributed to advancing the use of PI for several fresh horticultural commodities. However, more research needs to be done to evaluate factors that may reduce the efficacy of PI treatment schedules to increase PI acceptance and application. Low oxygen levels before and during irradiation treatments have the potential to reduce the efficacy of PI for some insect pests. Modified atmospheres (MA) and controlled atmospheres (CA) with low oxygen levels are commonly used to impede senescence and preserve the quality of fresh fruits and vegetables. Thus, it is critical to evaluate whether postharvest practices used to preserve the quality of fresh produce, such as MA and CA, can reduce the efficacy of PI targeting insect pests, particularly tephritid fruit flies due to their diversity, geographical distribution, and great economic importance in the agricultural sector.

Previous work has shown that PI treatments below the recommended doses and under low oxygen conditions can actually increase the radiotolerance of some tephritids, drosophilids, lepidopterans, and coleopterans. As a result, international organizations and regulatory agencies either recommend or impose restrictions on the application of PI for horticultural commodities maintained under modified atmospheres with low oxygen levels. For example, the International Plant Protection Convention (IPPC) encourages the restriction of PI treatments targeting insect pests for commodities stored in modified atmospheres, except for *Rhagoletis pomonella* and *Grapholita molesta*. Meanwhile, USDA APHIS imposes restrictions on irradiation treatments for commodities stored in packaging or other conditions with oxygen levels below 10%.

Despite the radioprotective effect of low oxygen treatments during low dose irradiation in tephritid fruit flies, it is unclear whether this protection persists at radiation doses used for phytosanitary treatments. In addition, most studies on this topic have focused on the effect either very low levels of oxygen or Modified Atmosphere Packaging (MAP) have on the efficacy of phytosanitary irradiation against a few fruit fly species. Thus, the use of a consistent methodology in studies comparing multiple tephritid species is essential to evaluate the extent to which low to moderate oxygen levels, before and during irradiation, can affect the survival and development of these species. This study simultaneously evaluated the effect of normoxia (normal air: ~21% O_2, 0% CO_2), hypoxia (~5% O_2, ~15% CO_2), and severe hypoxia (~0.3% O_2, ~21% CO_2) on the efficacy of phytosanitary irradiation for *Anastrepha fraterculus*, *A. ludens*, *Bactrocera dorsalis*, and *Ceratitis capitata* using the same methodology and approach.

Methods

Fruit fly colonies were maintained and experiments were carried out at the Insect Pest Control Laboratory of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture at Seibersdorf, Austria. Naturally infested mangoes and mandarins were used in the experiments. Mangoes were infested by either *A. fraterculus* or *A. ludens* (Figure 1) for up to six hours, and mandarins were infested by either *B. dorsalis* or *C. capitata* for up to two hours. All fruits were sanitized with an antifungal solution (4% sodium benzoate and 1% sodium hypochlorite) before and after infestation.

Before irradiation, infested mangoes were individually placed into plastic chambers (13.0 cm diameter × 18.5 cm high) under normoxia, hypoxia, or severe hypoxia conditions. Similarly, two infested mandarins were placed in a plastic chamber (12.5 cm diameter × 19.5 cm high) and were maintained under the same three atmospheric treatments.

Before gas flushing and irradiation, three pieces (1 cm x 1 cm) of GAFCHROMICTM HD-V2 dosimetry films were positioned below and above each mango or mandarin to measure the absorbed radiation dose at different positions. Plastic chambers containing mangoes or mandarins infested with third instars were flushed for three minutes with certified gas mixtures for hypoxia (nitrogen: 78%, oxygen: 5%, carbon dioxide: 16%, argon: 1%) and severe hypoxia (nitrogen: 78%, oxygen: 0%, carbon dioxide: 21%, argon: 1%) treatments. After flushing the plastic chambers, the concentrations of oxygen and carbon dioxide were measured hourly using a CheckMate 3 gas analyzer (Figure 2).

Figure 1. *Anastrepha ludens* oviposits into a mango as fruit are infested before treatment.

Infested mangoes and mandarins were kept under low-oxygen atmospheres (hypoxia or severe-hypoxia) for six hours or under ambient air (normoxia) before irradiation with gamma rays.

Figure 2. Measurements of O_2 and CO_2 levels were taken from containers holding infested fruits.

Infested fruits treated under normoxia, hypoxia, and severe hypoxia conditions were irradiated using a Gammacell 220 with dose rates ranging from 75 to 95 $Gy.min^{-1}$ (uncertainty of 1.9–5.9 % at 95% CL). The nominal doses tested ranged from non-lethal doses to lethal doses used as PI treatments. Briefly, mangoes infested with either *A. fraterculus* or *A. ludens* third instars were irradiated with the non-lethal doses of 25, 35, and 50 Gy or with the PI dose of 70 Gy. Mandarins infested with *B. dorsalis* third instars were irradiated with non-lethal doses of 30, 40, and 80 Gy or with the PI doses of 116 (pending approval) and 150 Gy. Mandarins infested with *C. capitata* third instars were irradiated with non -lethal doses of 20, 30, 50, and 70 Gy or with the PI dose of 100 Gy. Non-irradiated fruits (normoxia, hypoxia, and severe-hypoxia controls) were handled similarly to irradiated fruits.

Absorbed dose was verified using HD-V2 Gafchromic® film placed at three levels for mandarins (bottom, middle, and top) or two levels for mangoes (bottom and top). HD-V2 films were read through an optical density meter (DoseReader 4, RadGen®) 24 hours after exposure. After irradiation, mangoes and mandarins were incubated and dissected within seven days after treatment. The numbers of dead and live third instars and puparia were recorded. Treatment efficacy was determined by the prevention of adult emergence. Results for *A. fraterculus* and *A. ludens* are reported in Table 1 and results for *B. dorsalis* and *C. capitate* are reported in Table 2.

Conclusion

The results of this study suggest that hypoxic and severe-hypoxic conditioning before and during irradiation can increase the emergence rates of *A. fraterculus*, *A. ludens*, *B. dorsalis,* and *C. capitata* only at sub-lethal doses of gamma radiation. At approved PI doses, all third instars treated before and during irradiation with hypoxia or severe hypoxia failed to emerge as adults (Tables 1 & 2). Absorbed doses, oxygen, and carbon dioxide levels were systematically measured for all treated mangoes and mandarins. Based on those findings, regulatory agencies should evaluate restrictions applied to phytosanitary irradiation against tephritid fruit flies for fresh horticultural commodities stored under low oxygen levels.

Table 1. The number treated and adult survival (mean ± SE) for third instar *Anastrepha fraterculus* and *Anastrepha ludens* irradiated at nominal doses and atmospheric conditions.

		A. fraterculus		*A. ludens*	
Atmospheric conditions[1]	**Dose (Gy)**	**Number treated**	**% emergence[2]**	**Number treated**	**% emergence[2]**
Normoxia	0	8,976	75.8 ± 2.4	5,985	68.3 ± 3.1
Hypoxia		7,318	80.5 ± 2.3	3,757	81.3 ± 2.6
Severe hypoxia		6,475	70.6 ± 3.7	3,863	64.7 ± 4.5
Normoxia	25	6,287	2.2 ± 0.5	8,797	2.1 ± 0.5
Hypoxia		8,578	6.0 ± 1.3	5,539	9.3 ± 4.3
Severe hypoxia		7,145	38.7 ± 4.3	2,639	35.6 ± 5.4
Normoxia	35	2,483	0.2 ± 0.3	4,088	0.1 ± 0.1
Hypoxia		3,275	0.3 ± 0.0	2,864	0.1 ± 0.1
Severe hypoxia		2,701	5.5 ± 3.0	2,315	1.5 ± 0.9
Normoxia	50	2,224	0.0 ± 0.0	2,731	0.0 ± 0.0
Hypoxia		2,462	0.0 ± 0.0	2,996	0.0 ± 0.0
Severe hypoxia		2,318	0.0 ± 0.0	3,237	0.1 ± 0.1
Normoxia	70	5,501	0.0 ± 0.0	1,990	0.0 ± 0.0
Hypoxia		5,911	0.0 ± 0.0	2,468	0.0 ± 0.0
Severe hypoxia		6,896	0.0 ± 0.0	2,435	0.0 ± 0.0

[1]Normoxia (~21.0% O_2, 0.0% CO_2), hypoxia (5.5 ± 0.1% O_2, 15.7 ± 0.2% CO_2) and severe hypoxia (0.3 ± 0.02% O_2, 22.2 ± 0.2% CO_2).
[2]Emergence rates were adjusted using Abbott's correction.

Table 2. The number treated and adult survival (mean ± SE) for third instar *Bactrocera dorsalis* and *Ceratitus capitata* irradiated at nominal doses and atmospheric conditions.

		B. dorsalis			*C. capitata*	
Atmospheric conditions[1]	**Dose (Gy)**	**Number treated**	**% emergence[2]**	**Dose (Gy)**	**Number treated**	**% emergence[2]**
Normoxia	0	18,397	84.7 ± 1.5	0	5,901	78.3 ± 2.5
Hypoxia		4,050	82.4 ± 2.7		1,004	74.8 ± 3.1
Severe hypoxia		11,168	81.0 ± 2.2		2,462	77.6 ± 3.6
Normoxia	30	1,172	5.5 ± 1.8	20	529	10.5 ± 5.6
Hypoxia		4,523	14.4 ± 3.6		977	29.1 ± 6.2
Severe hypoxia		1,508	45.9 ± 5.6		1,320	67.3 ± 6.0
Normoxia	40	4,899	3.8 ± 1.1	30	2,590	3.6 ± 1.6
Hypoxia		4,523	12.0 ± 3.2		543	12.2 ± 6.0
Severe hypoxia		3,820	21.8 ± 3.8		1,053	19.8 ± 5.2
Normoxia	80	2,289	0.01 ± 0.01	5	654	0.6 ± 0.6
Hypoxia		3,699	0.0 ± 0.0		1,248	0.2 ± 0.2
Severe hypoxia		1,069	0.0 ± 0.0		880	0.0 ± 0.0
Normoxia	116	6,405	0.0 ± 0.0	70	1,031	0.0 ± 0.0
Hypoxia		4,967	0.0 ± 0.0		2,727	0.0 ± 0.0
Severe hypoxia		4,511	0.0 ± 0.0		1,334	0.0 ± 0.0
Normoxia	150	6,175	0.0 ± 0.0	100	2,969	0.0 ± 0.0
Hypoxia		1,852	0.0 ± 0.0		2,138	0.0 ± 0.0
Severe hypoxia		3,938	0.0 ± 0.0		1,550	0.0 ± 0.0

[1]Normoxia (~21.0% O2, 0.0% CO2), hypoxia (5.3 ± 0.04% O2, 15.0 ± 0.1% CO2) and severe hypoxia (0.3 ± 0.02% O2, 21.6 ± 0.1% CO2).
[2]Emergence rates were adjusted using Abbott's correction.

Khapra beetle survey at distillers' dried grain production facilities at risk of infestation

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Introduction

Khapra beetle, *Trogoderma granarium*, is a pest of a wide range of stored products that has expanded well outside of its native range in south-central Asia. Adapted to warmer environments, khapra beetle can generally thrive outdoors in tropical and subtropical areas; however, it also has the potential to create severe problems for agricultural production systems in temperate regions. Low relative humidity and high temperature, at or above 35°C, can lead to rapid outbreaks [1]. At the same time, larvae can go into diapause when stressed by starvation or cooler temperatures, persisting for long periods until environmental conditions become more favorable. This makes the insect easily adaptable to heated indoor storage facilities, where control efforts can be thwarted by the reemergence of diapausing larvae [2].

Because of global quarantine restrictions, the presence of an established khapra beetle population would not only threaten the quality of stored food products but also access to export markets that do not have the pest. The pest is not established in the United States but is frequently intercepted during customs inspections. Depending on the scale of the introduction, remediation procedures can vary substantially and be very expensive [3].

In early 2019, it was reported that khapra beetle had been found in shipping containers that had an association with two production facilities for distillers' dried grain with solubles (DDGS) in Illinois. A rapid response was mobilized to determine if the pest was located at either of these locations. The response to the event allowed the formulation and establishment of protocols for such emergency events.

The results of the trapping portion of the response in Illinois will be used to refine response to similar events in the future. While no khapra beetle were found, many specimens of the related dermestid warehouse beetle, *Trogoderma variabile*, were found. In addition to the traps used for APHIS surveys, another commercially available trap was also used. We were, therefore, able to assess the advantages of both traps for future events. The exercise also allowed us to assess the resources required at the different stages of an emergency response and the need for access to synergistic methods, such as molecular identification.

Methods

In January of 2019, USDA personnel from Federal Grain Inspection Service, and APHIS-PPQ met in Illinois at two locations where DDGS are produced for international exports to determine if the facilities may have been exposed to khapra beetles via shipping containers. The team inspected the premise and instituted a six-month-long monitoring program to ensure a khapra beetle population was not at either facility.

The personnel toured the facilities, identifying areas where khapra beetle would be more likely to find food or refuge, such as grain storage piles, cracks in walls, and conveyance structures. Sweepings of grains from the floors of the facilities were collected and examined for khapra beetles, and traps were placed at indoor locations. The tour took place during an extreme weather event, where the low temperature reached approximately -29°C. It should be noted that the extreme temperatures would have certainly killed all khapra beetle in outdoor DDGS storage locations. However, as a safeguard, it was decided that outdoor trapping would also begin in April when seasonal insect activity was likely to commence.

The two traps used were the Insect Limited™ All Beetle trap kit with Pheromone Gel Attractant and Storgard APHIS KIT-KB wall trap (Figure 1). The Insects Limited™ trap is a loosely standing floor trap, while the Storgard trap mounts on the wall. Both traps include kairomone and pheromone odors that are known to attract adult and larval khapra beetles [4].

Figure 1. Traps deployed at distillers' dried grains with solubles production facilities included an Insect Limited™ floor trap with pheromone gel (left) and a Storgard APHIS KIT-KB wall trap with wheat germ and pheromone lure (right).

There were six floor traps and ten wall traps placed at the first, smaller facility (DDGS Producer 1). Nine floor traps and 11 wall traps were placed at the second, larger facility (DDGS Producer 2). It was ensured that at least one of each trap type was placed in each room considered to be at risk for khapra infestation. Traps were also placed within and near semi-enclosed outdoor grain pile storage. There were also some larger open areas within the facilities where multiple traps were placed. Within each of the three facilities, three different floor sweepings were collected from different areas where loose grain had accumulated.

The traps were checked at two-week intervals. The wheat germ contents of the wall traps, which were most likely to contain the pests, were removed and replaced. The floor traps were also checked biweekly, with the gel bait replaced at one-month intervals since many insects of various taxa enter the floor traps and die in the liquid gel contents. The manufacturers' recommendations for these products were followed at both intervals. Floor sweepings and the contents of each trap were sent to APHIS's National Identification Service (NIS). Monitoring was extended for approximately six months, ending in August 2019 to ensure adequate coverage of the warmer months when outdoor populations could thrive.

Results

When evaluating the facilities, the team of investigators did not find any evidence of khapra beetle infestation in cracks or corners of buildings. These locations were pointed out to plant personnel for continued monitoring. The APHIS-PPQ Plant Health Safeguarding Specialist assigned to check and maintain the traps at these locations confirmed that no evidence of dermestid activities was observed in such hidden locations. Among the six floor sweepings initially collected, five were negative for Dermestidae. One sweeping from the warehouse of the first DDGS producer had three larval dermestid exuvae that were determined by NIS not to be khapra beetle.

All trap captures were morphologically confirmed to not be khapra beetle by NIS. Most were tentatively identified as warehouse beetle. When the pattern of catches across the six-month monitoring window was examined it was clear that there were differences among the trap type and site combinations (Figure 2). Captures in the floor traps were much more sporadic, occurring at certain dates at high abundances, such as in the middle of the sampling period at the first site (Figure 2A). Furthermore, there was a peak in captures earlier in the monitoring period in April at the second facility that was not observed elsewhere (Figure 2C). The gel in the floor trap causes the insects to drown, along with bycatches of other insects. It is thus possible that the odor emitted from the dying insects may be repellent to dermestids.

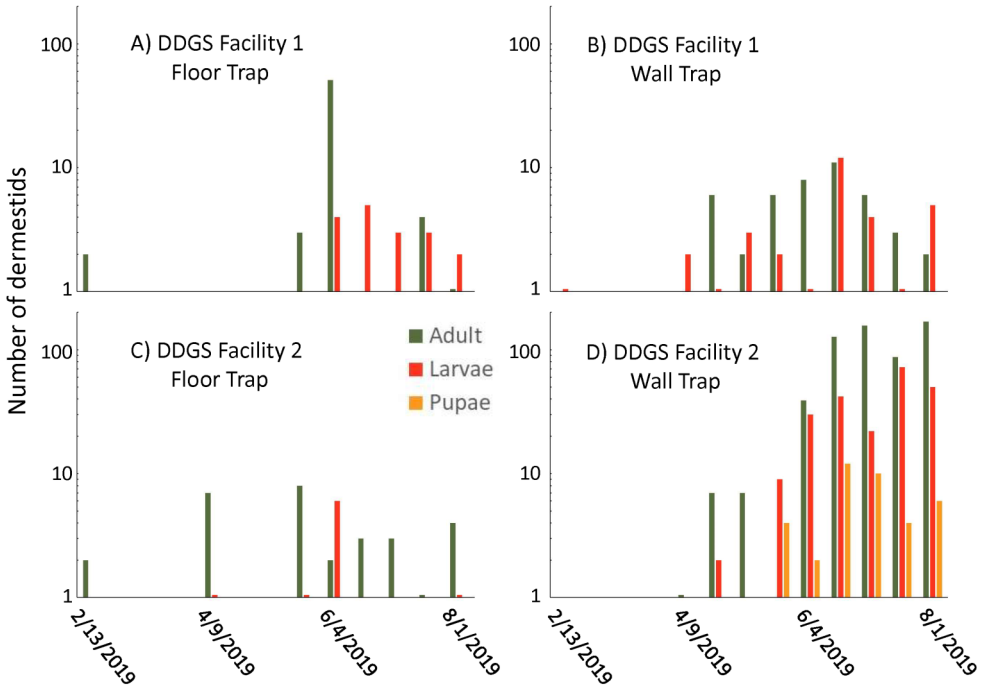

Figure 2. Pooled number of adults, larvae, and pupae in biweekly catches of each trap type at the two monitored Distiller's dried grains with solubles production facilities. The number of traps placed at each location is provided in Table 1.

Table 1. Characteristics of dermestid trap capture at Distiller's dried grains with solubles facilities monitored in Illinois.

Facility	DDGS 1		DDGS 2	
Trap type	Floor	Wall	Floor	Wall
Number of traps deployed	6	10	9	11
Number of Dermestids caught	78	76	39	857
Percentage of adults captured	78.2	57.9	76.9	70.1
Percentage pupae captured	0	0	0	4.0
Percentage of seasonal detection/trap	100	100	66.7	100
Number of detection events/season	14	30	30	39
Percentage of larvae-only/detection events	42.9	36.7	10	7.7

More consistent captures were made in the wall trap at both locations. However, it was apparent that more captures occurred at the second site (Table 1, Figure 2). Furthermore, the wall traps at the second site were the only to capture pupae. Overall, the pupae captures were only 4% of the total in these traps (Table 1).

Because the wall traps are baited with wheat germ, which allows the insects to remain alive and feed, it cannot be confirmed whether the stages observed upon collection were the same as those that entered the trap. However, the collection period was only 14 days, making it impossible for an entire life cycle to be completed. Therefore, the pupae observed must have entered the traps as larvae and pupated in the traps.

When considering the detection rate of all traps, it was apparent that any of the traps would positively indicate a dermestid at some point in the monitoring window with an equal probability. This rate was around 100% for each trap and location combination, except only two-thirds of the floor traps at the second site caught beetles (Table 1). It was also noted that sometimes larvae without accompanying adults were found during detections. Such detections occurred more often at the first site (~40%) than the second site (~10%); this variable was not affected by trap type (Table 1). This observation provides further evidence that larvae will indeed crawl into both trap types readily.

Conclusion

The wall traps displayed several advantages that suggest they are the most effective trap for rapid response events. Unlike floor traps, the wall traps were able to detect the presence of larger dermestid populations at the second site.

The wall traps also were able to detect *T. variabile* larvae more efficiently than the floor traps, which may be beneficial for khapra beetle detection, considering that larva tend to remain in diapause. Beyond these advantages, the wall traps are also easier to deploy and service, whereas floor traps can easily be stepped on, swept up, or damaged if care is not taken.

The large number of adult *T. variabile* captures in wall traps became overwhelming to identifiers and it is possible that adding hanging traps baited with pheromone may have eased this burden as *T. variabile* can fly, unlike khapra beetle. Therefore, hanging traps may have diverted flying *T. variabile* adults away from the wall and floor traps, allowing identifiers to rule out dermestids caught in hanging traps as possible khapra beetle.

However, hanging traps would not divert *T. variabile* larvae from the wall and floor traps targeting khapra beetle. The two species are much more difficult to distinguish morphologically at the larval stage. Even if all the adult *T. variabile* could have been diverted to hanging traps, it would require several weeks for identifiers to determine that none of the larval specimens were khapra beetle. Thus access to inexpensive, fast, and reliable molecular tools for dermestid identification is needed to improve the response to such threats to agricultural production facilities from khapra beetle.

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Impact of insecticides and oils in preventing hatch from spotted lanternfly egg masses

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Introduction

Spotted lanternfly, SLF, *Lycorma delicatula*, a sap-feeding insect native to Taiwan and China, was first detected in Pennsylvania in 2014. This invasive pest has since spread to over a dozen counties in southeastern Pennsylvania, and counties in New Jersey, Delaware, Maryland, and Virginia that border or are associated with the Pennsylvania infestation. There is great potential for this insect to spread beyond its current boundaries via dispersal or as a result of its egg-laying habits. SLF adults can lay eggs on almost any flat surface including not only the bark of their preferred host tree but also lawn furniture, rocks, fence posts, cut logs, firewood, and other similar substrates.

Though there is little information available on SLF egg mass control, Shin et al. tested various products from six insecticide classes and found that chlorpyrifos resulted in 94% or greater mortality of SLF hatching from treated egg masses [1]. Additionally, far less control was observed when field-collected egg masses were treated in May, right before hatch. The other insecticides and treatments tested showed much lower levels of efficacy with corrected mortality ranging from 0 to 37%. However, it should be noted that only two to three egg masses were used to assess each treatment.

As a result of the limited information on SLF control in the scientific literature, additional testing for a regulatory treatment that could prevent or drastically reduce SLF hatch would be valuable for population suppression. It would additionally serve as a key regulatory tool, allowing for the safe movement of commodities, nursery stock, household articles, rail cars, and vehicles outside of the quarantine zone.

Methods

Over the last two field seasons, our laboratory tested seven pyrethroid insecticides and two dormant oil sprays to determine if surface treatments would be an effective means to reduce or eliminate egg hatch. Separate fall (2018) and spring (2019) applications were also carried out to examine if timing of application had an effect. Refer to Table 1 for rates used and products tested.

Table 1. Treatment and rate information of products tested on SLF egg masses in the field in Pennsylvania. Twenty to thirty-one egg masses per product were exposed via a simple surface treatment using a hand-held sprayer. Asterisks indicates that the higher rate was tested in 2019.

Product name and manufacturer	Active ingredient	EPA #	Chemical class	Rate	Year assessed	
					2018	2019
Talstar P — FMC Corp	bifenthin 7.9%	279-3206	synthetic pyrethroid	0.06% & 0.12% dilutions	√	√*
Tempo SC Ultra — Bayer	B-cyfluthrin 11.8%	432-1363	synthetic pyrethroid	0.025% & 0.05% dilutions	√	√*
Demand CS — Syngenta	lambda cyhalothrin 9.7%	100-1066	synthetic pyrethroid	0.04% dilution	√	
Onslaught — MGK	esfenvalerate 6.4%	1021-1815	synthetic pyrethroid	0.05% dilution	√	
Chlorpyrifos 4E — Quali-Pro	chlorpyrifos 42.5%	66222-19	organophosphate	0.44% dilution		√
Orthene WSP — AMVAC Chemical Corp	acephate 75%	5481-8971	organophosphate	0.076 lbs per gallon of water		√
Sevin SL — Bayer	carbaryl 43%	432-1227	carbamate	0.11% dilution		√
Golden Pest Spray Oil — Stoller Enterprises, Inc	soybean oil 93%	57538-11	vegetable oil	50% dilution	√	√
Horticultural & Dormant Oil — Bonide Products, Inc	mineral oil 98%	4-80	petroleum oil	3% & 7% dilutions	√	√*

Individual egg masses (Figure 1) were identified from the lower bole of host trees and randomly assigned to treatment groups. Egg masses were sprayed to run-off using a 24-ounce hand pump sprayer, allowed to dry, and transported back to an incubator to monitor hatch in the Otis Insect Containment Facility. Egg masses were maintained in the incubator at 25°C, 65% humidity, 16:8 light cycle, and checked regularly until the first hatch was observed about three weeks later. Hatch was monitored daily and mortality assessments were made for three weeks post-hatch.

Figure 1. Nymphs emerging from a treated egg mass.

Results

Initial treatments in spring of 2018 showed that while the pyrethroid products suppressed emergence to varying degrees, the Golden Pest Spray Oil was the most successful at preventing emergence of viable SLF nymphs. However, this trial was marred by poor emergence among the egg masses in the untreated group. In fact, the control group had lower emergence numbers than some of the chemical treatments. Full details of the methods and results can be found in the 2018 Otis Annual Report [2]. This directed us to conduct the additional testing that is outlined here, where application rates were increased, and the most promising products, including Golden Pest Spray Oil were retested.

Due to limited availability of SLF egg masses in the field, we had to limit our treatment choices for fall 2018. We tested a double rate of the most promising pyrethrin product (Talstar), the two horticultural oils (Golden Pest Spray Oil and Bonide), and an untreated group; 20-25 egg masses were tested per group. Sufficient egg masses were procured for the spring 2019 application so that several additional products from three insecticide classes and the oils were tested together. Five insecticide products and the two horticultural oils were tested against an untreated group (29-31 egg masses per group). Following the spring applications, all egg masses, both fall 2018 and spring 2019, were chiseled off of trees and transported to the Otis Insect Containment Facility and monitored for emergence.

For the fall 2018 treatments, over 400 SLF nymphs hatched from the untreated group, a notable improvement compared to the 47 nymphs that hatched from the spring 2018 untreated group. Nymphs did not hatch from the Talstar treatment and the two oil treatments provided significant levels of control, preventing between 82-88% of the nymphs from emerging (Figure 2).

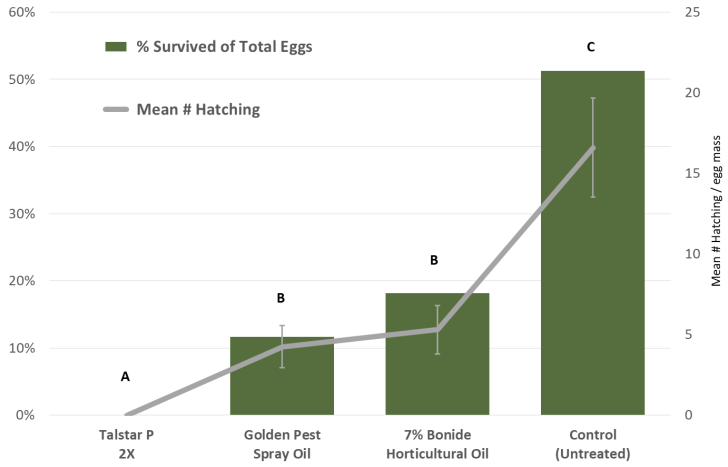

Figure 2. Egg masses were surface treated in the fall of 2018 with various products. ANOVA test of mean hatch data (±SE) shows significant differences between treatments; treatments with similar letters are not statistically different from each other at $p = 0.05$.

Egg hatch from the spring 2019 untreated group was good and, similar to the fall 2018 group, about 27% of egg masses failed to produce any nymphs. Several treatments significantly increased the likelihood that nymphs would not hatch from the egg masses. Golden Pest Oil and chlorpyrifos prevented 90% and 96%, respectively, of egg masses from producing nymphs ($F_{7,238} = 17.74$, $p < 0.001$). While Bonide and Talstar treatments doubled the chance for zero emergence, there was not a statistical difference from the control group for either treatment.

In contrast to the fall 2018 application of Talstar, quite a few nymphs hatched from the spring 2019 Talstar treatment. The two oil treatments did well again, yielding between 93-98% control (Figure 3). Very little to no emergence was seen in the Tempo and chlorpyrifos treatments and all five of these treatments had significantly lower numbers of SLF nymphs hatching per egg mass compared to the Orthene, Sevin, and control treatments (Figure 3).

Discussion

The emergence of SLF nymphs from their winter egg masses can be impacted by a simple spot spray application that soaks the egg mass. The best results were found when using the highest labeled rate (Structural Pests) of Tempo SC Ultra and the 'Outdoor Surfaces' rate on the Chlorpyrifos 4E label. A high level of control was also obtained when using Talstar P (Structural Pest rate) and the two horticultural oils. Spot applications of Golden Pest Spray Oil and Chlorpyrifos 4E resulted in a very high percentage of egg masses with zero hatch success (90.0% and 96.4%, respectively).

The use of any of these products and rates for spot treatment of SLF egg masses must first be approved by the local state department of agriculture. Treating SLF egg masses in the fall did not appear to make a difference in the success of nymph hatch. The two horticultural oils trended toward better control with a spring application as compared to the fall, whereas complete control was seen with the Talstar P formulation applied in the fall but resulted in 7% survival with a spring application. Egg masses treated in the spring of 2019 were harvested one day following treatment. It is unclear if leaving the treated egg masses in the field for a longer period impacts hatching success.

The lack of emergence from a significant portion of egg masses may be due in part from winter kill or other biological factors. Percent hatch from egg masses in the control group ranged between 51.2% to 59.0%, with SLF nymphs failing to hatch from between 24.0-26.7% of the egg masses. The poor hatch of field-collected egg masses has been observed by others and hatching success was noted to vary based on where oviposition took place [2].

Findings from this study will enhance the currently available information on SLF control and provide additional tools that the public as well as state and federal officials can use to help suppress SLF populations. The results presented here can also inform regulatory treatment options for the safe movement of articles in and out of the quarantine zones.

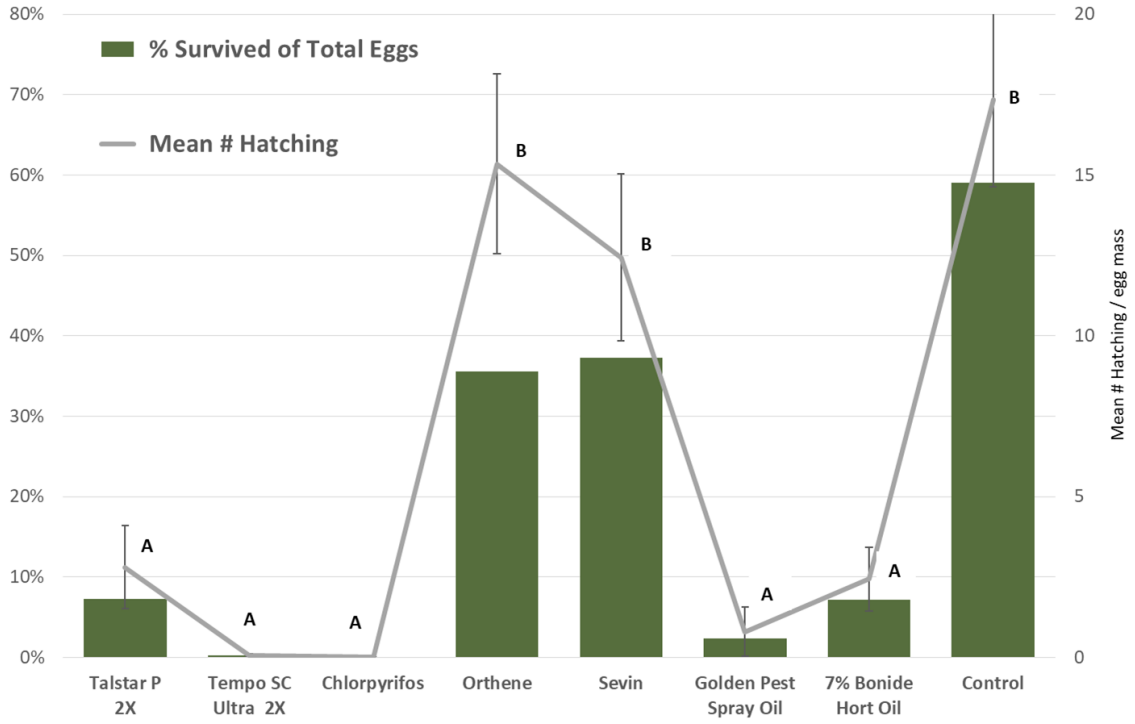

Figure 3. Egg masses surface treated in the spring of 2019 with various products. ANOVA test of mean hatch data (±SE) shows significant differences between treatments; treatments with similar letters are not statistically different from each other at $p = 0.05$.

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Utilization of trees of *Platanus* species by the Asian longhorned beetle

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Introduction

Since the detection of the Asian longhorned beetle, ALB, *Anoplophora glabripennis*, in the United States in 1996, great efforts have been made by federal and state agencies to eradicate the beetle. Understanding the host range and preference of the beetle is critical to optimize survey efforts and prophylactic treatment of host trees. Current knowledge of ALB's host range has been developed through surveys in China and laboratory testing in the U.S. Our efforts have primarily focused on trees considered to be occasional or rare hosts as described in the APHIS ALB host list (Wang, 2015). A tree species is only considered an ALB host when ALB development can be completed on the tree from egg to adult emergence. Evaluation of additional species is necessary to better understand the utilization of occasional and rare hosts by ALB. For example, the New York ALB program has reported finding ALB exit holes on London planetree, *Platanus* × *acerifolia*, however, no exit holes have been found to date in surveys of the congeneric species American sycamore, *Platanus occidentalis*. We report here a summary of findings from the evaluation of selected *Platanus* species as an ALB host through surveys in different provinces of China and tests in common garden settings.

Surveys in China

Surveys were conducted from 2007-2018 in several locations in China to evaluate the utilization of *Platanus* and other tree species by ALB (Figure 1). Sites with at least moderate ALB population density, defined as over 50% ALB infestation of a known primary host such as willow or maple, were selected for surveys. The surveys were conducted from May to October, when signs of infestation such as exit holes, frass, or leaf/twig feeding by adult beetles can easily be detected. Three *Platanus* tree species were identified as potential rare hosts, warranting further investigation in the survey including: London planetree, American sycamore, and the Oriental planetree, *P. orientalis*.

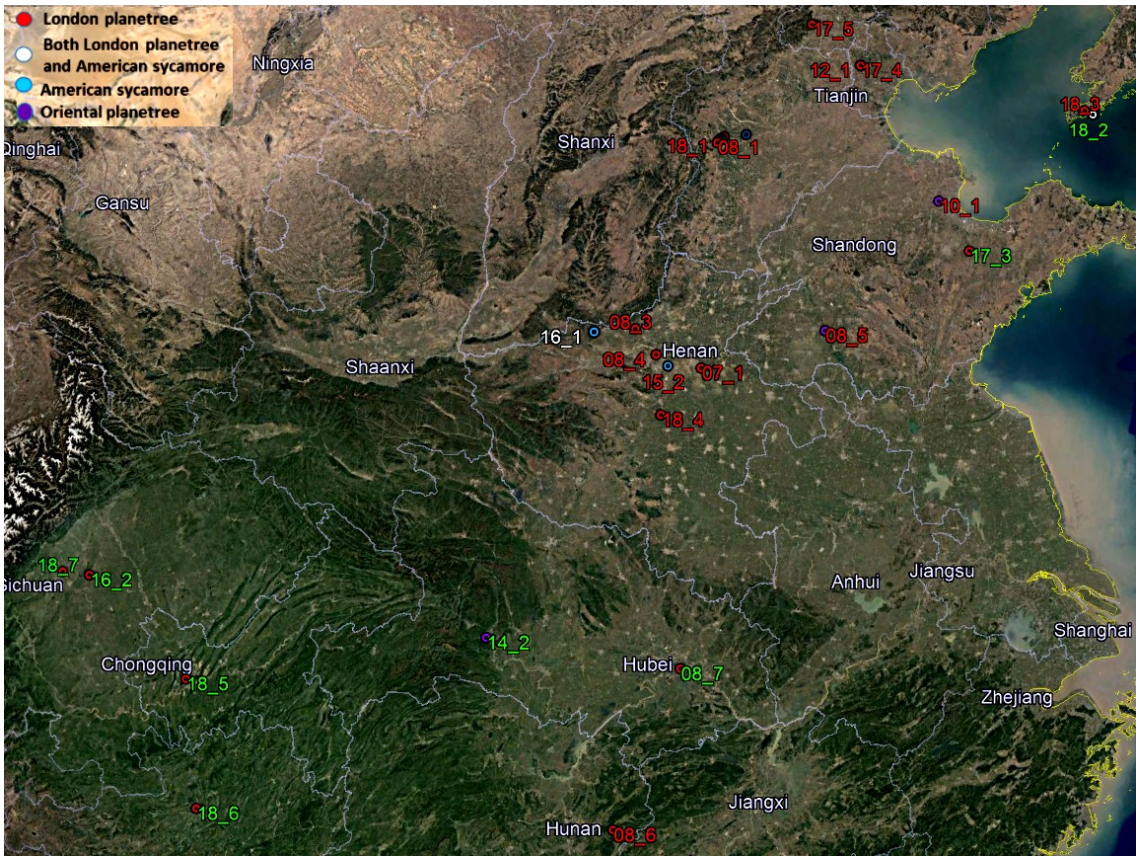

Figure 1. Survey results of the infestation of *Platanus* species by Asian longhorned beetle (ALB) at different sites in China. The labels indicate: red = ALB; green = no ALB found. The first digit represents the year trees were surveyed, for example, 08 stands for 2008 and 18 stands for 2018, while the last digit of the label stands for the site ID in that year.

Through surveying, we observed that ALB infest London planetree, and in some cases adults emergenced. In 2007, at a survey site in Kaifeng, Henan province of China, a total of 31 exit holes were found on 31 London planetrees from May to July. Six infested branches and trunks were removed and subsequently caged. Three ALB emerged in mid- to late-July. During the survey, additional observations of infestation were noted such as, ALB adults making egg pits and frass being deposited from branches and the main trunk of the trees.

A few ALB exit holes were observed in American sycamore, which is a less common roadside tree in China. Oriental planetree, another uncommon street tree, was found to be infested by ALB during the survey at most sites. However, *Platanus* trees were not infested by ALB at some sites we surveyed, although ALB were seen emerging from elm or willow trees nearby.

Choice and non-choice tests in Beijing, China

Studies were mainly conducted in common garden settings where potential ALB host trees were transplanted alongside known host trees. Two types of tests were performed: choice and non-choice. Choice tests were conducted from 2009-2010, and non-choice tests were conducted in 2011 and then again from 2017-2019.

2009-2010 choice-test with London planetree

This test was conducted to compare ALB's utilization of London planetree against four species of ash trees. A large screen-house was built to cage five tree species, with 5-10 trees representing each species for choice-test evaluation. The five species included 1) London planetree, 2) white ash, *Fraxinus americana*, 3) green ash, *F. pennsylvanica*, 4) Arizona ash, *F. velutina*, and 5) Chinese ash, *F. chinensis.*

All species were considered rare or occasional hosts at the time of testing. Beetles were released three times between late June and mid-August of 2009 at 9-16 evenly distributed points in the screen-house. Between 100 and 180 marked adult beetles (approximately equal number of males and females) were released each time. Tree trunks and branches were checked periodically for eggs and active egg sites (determined by the presence of frass, indicating larval activity) as well as adult emergence for at least two years.

ALB laid more eggs in the London planetree than any of the other species, although the number was quite low (Table 1). No eggs were laid in Arizona ash. The number of observed active egg sites was lower for London planetree than for green ash. Beetle emergence was not observed on any London planetree from 2009-2010, while one beetle emerged from a green ash tree. The results of this test indicate that while ALB laid eggs on London planetree and three eggs hatched into larvae, none were able to complete full development. This incomplete development may be due to natural enemies since the survey results above indicate that London planetree is an ALB host.

2011 non-choice-tests

Paired male and female beetles were caged with tree sections that included portions of the trunk, as well as twigs and leaves for adult feeding.

Table 1. Utilization of London planetree and ash trees for egg-laying and beetle development.

Tree species	Total no. of eggs	Active egg sites in first 2-3 months	Larval activity before first adult emergence	No. of adults emerged in 2 years
London planetree	66	3	Not observed	0
White ash	0.4	0	Not observed	0
Green ash	57	29	2	1
Arizona ash	0	0	Not observed	0
Chinese ash	0.2	0.5	Not observed	0

The five species were 1) London planetree, 2) paper birch, *Betula papyrifera*, 3) Norway maple, *Acer platanoides*, 4) mono maple, *Acer mono*, and 5) boxelder, *Acer negundo*. All species, except for London planetree, were considered primary ALB hosts. After three weeks, caged tree sections were checked for egg pits, active sites, exit holes, and egg sites made by females. A portion of egg sites were dissected three months after caging to determine whether the egg sites were empty or contained ALB eggs or larvae. Additionally, beetle emergence was monitored periodically for two to three years if larval activity was observed.

London planetree had the lowest percentage of egg sites containing either an egg or a larva. Larval survival was the lowest on London planetree and the highest on boxelder (Table 2). Few egg sites were left after the dissections, so it was not possible to compare beetle emergence among the trees in the study.

2017-2019 non-choice test with American sycamore

To confirm the host potential of American sycamore, one pair of beetles was caged with sections of tree trunk (DBH 24.1 ± 6.7 cm) along with twigs and leaves of American sycamore. Six weeks after caging, the number of egg sites per cage was 12.0 ± 5.9, and the percentage of active sites was 54.7±38.4, indicating high variation among individual beetle pairs and trees, although more than half of the egg sites had larval activities. In total, three months after caging, 32 active egg sites were identified, indicating 32 eggs hatched into larvae. The percentage of active egg sites decreased to 40.8 ± 44.8% one year later in late June 2018, and by October 10, 2018, only two active sites were observed. A female adult emerged on July 4, 2019, while observations of the other active site indicated that frass was no longer being pushed out when checked on July 26, 2019. When the larval site was dissected in August 2019 no live larvae were found in the tunnel. We speculate that any remaining larvae may have been lost to predation. Although only one of the initial 32 larvae completed development to adult, this result confirms that ALB can utilize American sycamore as a complete host.

Conclusion

Asian longhorned beetle was able to infest all three *Platanus* species in China, although *Plantanus* is not as preferred or suitable a host compared to primary hosts such as maple and paper birch. A few ALB were able to complete development on London planetree in the survey conducted in China and on American sycamore in both the survey as well as the caged study, suggesting that both should be considered an occasional host. The reproductive success of ALB on *Platanus* trees appeared to be much lower than on the primary host tested. However, in the event that some *Platanus* trees are infested by ALB, they may serve as a reservoir if they are not detected.

Reference

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Table 2. The rate of egg hatch of Asian longhorned beetle and survival on London planetree and primary hosts.

Species	Egg sites per cage at 3 weeks	% of sites with egg or larva at 3 weeks	% of sites with live larva at 3 months
London planetree	22	37	13
Paper birch	16	47	33
Norway maple	16	82	59
Mono maple	54	54	26
Boxelder	31	87	87

Evaluation of battery-powered light traps for *Lymantria* species

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Introduction

The Asian gypsy moth, AGM, which includes two subspecies of *Lymantria dispar* and three closely related *Lymantria* species, are targets for survey and detection efforts as well as North American offshore AGM free vessel certification programs. Egg masses of other *Lymantria* species such as the casuarina moth, *L. xylina*, nun moth, *L. monacha*, and the rosy gypsy moth, *L. mathura*, have also been intercepted on vessels at U.S. ports. Surveillance and monitoring of these species currently rely on pheromone baited traps for males, who are generally much stronger fliers than the females. Most females of these species have been estimated to fly less than 1 km [1]. Therefore, male capture does not accurately represent risk levels for the immediate areas, since risk levels are primarily associated with females that lay egg masses. Although Wallner et al. has demonstrated that *Lymantria dispar asiatica*, LDA, are attracted to light, especially UV, no light traps have been developed for use in the AGM surveillance program [2]. With technological advancements in portable light sources and power supply as well as a better understanding of the biology and behavior of LDA and *L. xylina*, a portable light trap can now be developed as a potential survey tool.

We report here the results of our field bioassay of light traps for capturing female moths of LDA and casuarina moths. Two sites with moderate population levels of LDA and casuarina moth were selected for field bioassays of light traps. One site in northeastern China was selected to evaluate light trapping for LDA (site 1), and casuarina moth was evaluated at a site in southeastern China (site 2).

Light trap bioassay for LDA

The field site was located in the Erzhan forest farm in Heihe City, Heilongjiang province, China. To attract flying moths, LED lamps (12V/5W) with wavelengths of 365 nm (UVA light), and 420nm (UVV) were powered by 12V rechargeable motorcycle batteries to illuminate white fabric backgrounds (Figure 1). Each corner of the white fabric was fixed to two tree trunks using wires. Two lamps of each wavelength (UVV and UVA) were suspended about 2 m from the ground and separated by a distance of more than 30 m. The test began on July 26 and ended on July 27. On each day, the lights were turned on at 20:00 and the number of AGM male and female moths was counted at 21:00, 22:00 and 23:00, respectively.

Figure 1. Light trap for LDA adults at Erzhan forest farm in Heihe City, Heilongjiang province, China.

Moths were removed from the fabrics after each count was recorded. The results indicate that UVA light is far more attractive to both LDA males and females than the UVV light (Figure 2). Both UVA and UVV lights attract more males than females; approximately five times more with UVA and three times more with UVV. Nonetheless, a cumulative total of 30 female moths were found on the UVA illuminated fabrics during the study, indicating that the UVA LED light can be effective in detecting LDA females.

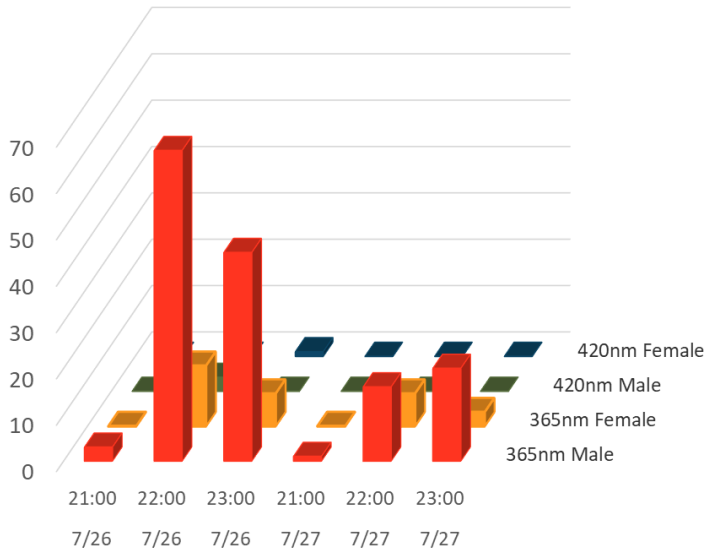

Figure 2. The number of LDA males and females present on white fabrics illuminated by lamps of two different wavelengths.

Light trap bioasays for casuarina moths

Field bioassays were conducted in *Casuarina equisetifolia* forests in Pingtan county, Fujian province, China to assess the effectiveness of light traps on casuarina moths. The battery powered light trap consisted of a hook, a top cap, a UV light tube surrounded by high voltage wire strings, and a bottom plastic jar for collecting insects. In the first test, light sources with the following wavelengths were selected: 320, 340, 350, 360, 365, 380, and 405 nm. LED (wavelength not measured), Longhao (365 ± 50nm), full-spectrum, and fluorescent (wavelength not specified) lamps were also tested as light sources. The traps with light tubes of differing wavelengths were placed along the edge of a casuarina forest between June 4 and June 7, 2019. The numbers of male and female casuarina moths in the individual traps were checked and recorded hourly from 19:00 to 4:00 for three consecutive days. The numbers of hourly and overall captures per trap were compared between traps with light sources of different wavelengths.

A total of 5,112 casuarina moths were captured during the study; however, only 15 were female (0.3%). Light traps with a wavelength of 360 nm caught the highest number of males, while traps with wavelengths of 350 nm and 365 nm caught the highest number of females (Figure 3). The majority (53.3%) of females were captured between the hours of 19:00 and 20:00, followed by the capture of 34% from 20:00-21:00. After 21:00, the number of females captured decreased significantly. No females were caught between 2300—2400. The largest number of males (29.7%) were captured between 23:00-0:00, followed by the next largest capture between the hours of 22:00-23:00 (Figure 4). The captures between 21:00-1:00 accounts for 83.3% of the total males collected.

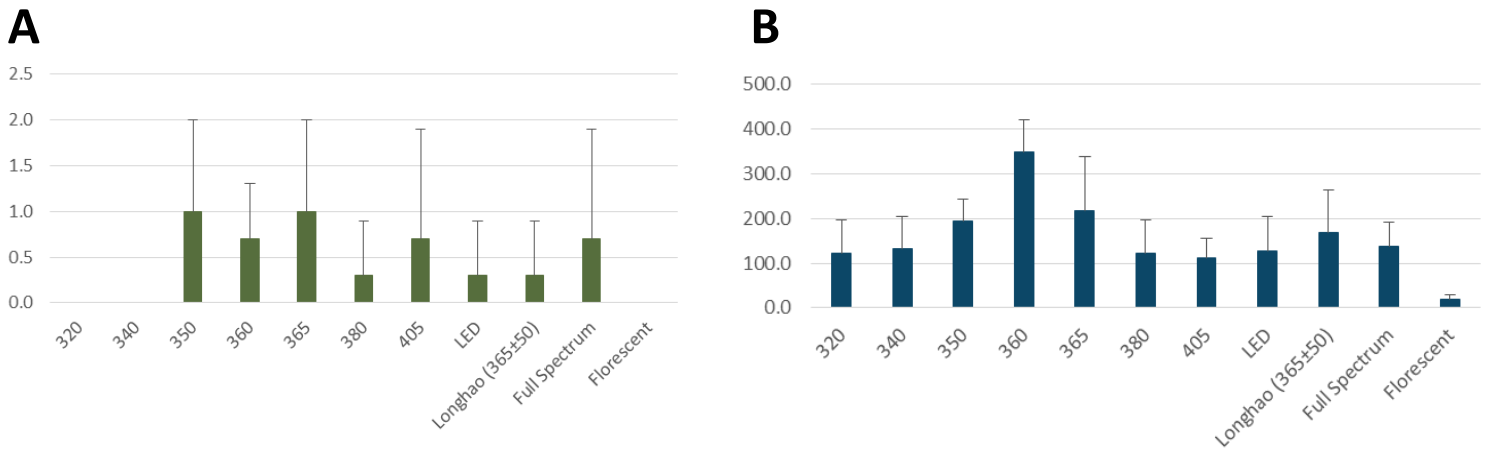

Figure 3. The number of A) female and B) male casuarina moths captured in traps illuminated with lights of different wavelengths.

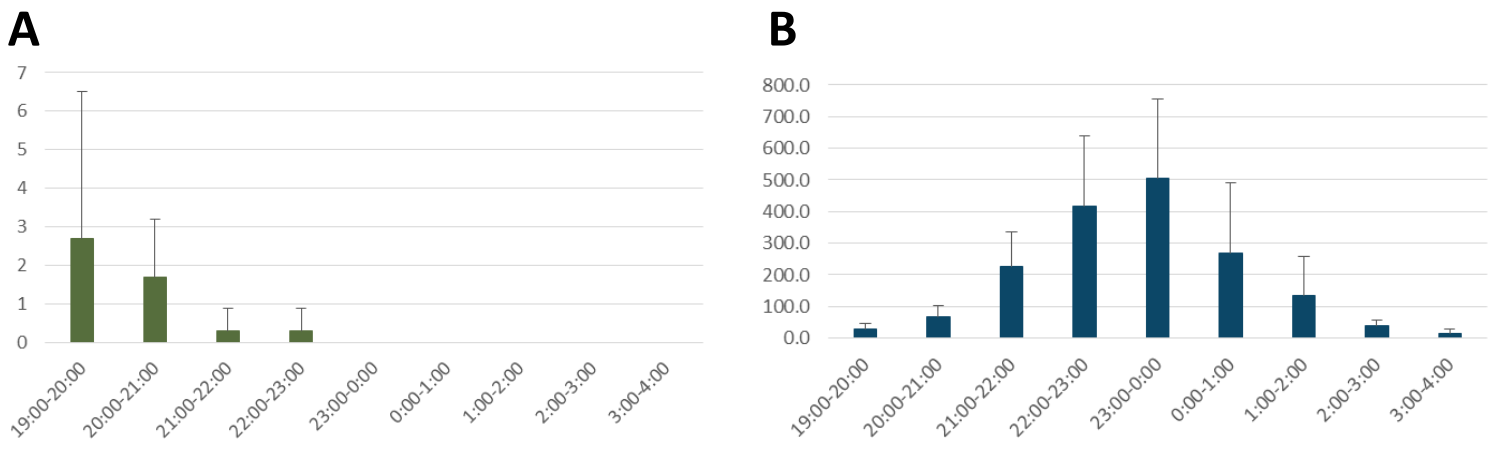

Figure 4. The number of A) female and B) male casuarina moths captured in the UVA light trap and pheromone trap.

Comparison of light traps and xylinalure baited traps

During a second experiment conducted in the same casuarina forest, light traps of 15 w and 360 nm wavelength (UVA) powered by portable batteries were installed alongside traps baited with xylinalure (cis-7,8-Epoxy-2-methyleicosane). Three light traps and three pheromone traps were alternatively placed. The test was conducted at two time intervals when the weather was relatively suitable for adult flying: June 7-8 and June 16-17. The air temperature ranged from 26-30°C for June 7-8 and 24-28°C for June 16-17; wind speed was measured to be around 3-8 m/s during both time frames.

A significantly higher number of casuarina moths were captured in the UVA light traps than the xylinalure baited pheromone traps during this field bioassay; although, trap capture varied significantly in both types of traps (Figure 5). The results suggest that the UVA light trap might be a better tool for monitoring casuarina moths.

Conclusions

In summary, the results from the field bioassays conducted at the two sites indicate that portable UVA light is a promising tool for trapping both male and female LDA. Additionally, our study suggest that UVA light could be a more efficient means for attracting the casuarina moth than the currently employed xylinalure baited pheromone trap. Attraction to cues such as pheromones and light are affected by environmental factors (e.g., weather conditions and time of day) and the biology of the target insect (e.g., mating status and age of adults). Additional studies that take into consideration some of these factors will be required to develop a feasible and effective portable UVA light trap. The risk of female moths laying egg masses on vessels is primarily related to the flight capacity of females and thus the distance of females to the vessel. We hope to be able to assess the efficacy of light traps at different distances for both species in our future field bioassays.

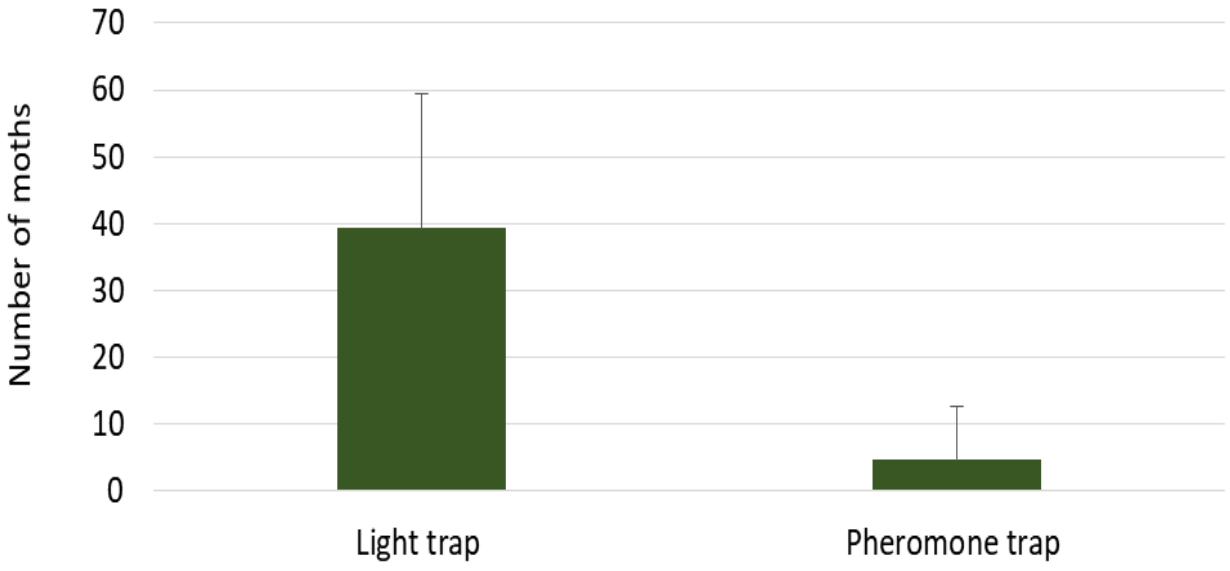

Figure 5. The number of casuarina moths found in the UVA light trap and xylinalure baited pheromone trap (per trap capture).

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Creation of a lending collection of 650 Ricker displays of federally quarantined insects

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Introduction

Due to the diversity of invasive insect species reared at Otis Laboratory, the facility's rearing group is frequently approached by state, federal, and other stakeholders who are seeking specimens and displays of invasive insects for training and outreach events. Outreach is an important function within APHIS, as it is critical for improving the public's capacity to recognize invasive insects and understand APHIS's efforts to prevent, detect, and control or eradicate invading populations. The PPQ Field Operations (FO) Outreach Coordinator recognized a great need for insect displays to serve as eye-catching items to help inform the public. The outreach coordinator reached out to Otis Laboratory with a proposal to develop a lending collection of insect displays that could be loaned to PPQ work units, State Plant Health Directors (SPHDs), and other PPQ affiliates for training and outreach. Otis Laboratory was eager to participate because creating a lending collection would support a more efficient use of resources while also enhancing PPQ's outreach tools. A one-year cooperative project was established to build a lending collection of insects from the federal list of quarantined species. The estimated need, calculated by the FO Outreach Coordinator, was 20 to 40 displays per species, based on prior records of simultaneous outreach and education events and on the number of states at risk or potentially at risk for introduction of each pest.

Developing the collection

Utilizing PPA 7721 (formerly Farm Bill) funding, a cooperative agreement was established between the rearing team at Otis Laboratory and the Plant Disease and Insect Clinic (PDIC) at North Carolina State University to create 650 Riker mounts of 18 species from the federal list of quarantined insects

Table 1. Invasive species, sources of specimens, and number of displays created for the outreach lending inventory.

Pest Name	Scientific Name	Insect Group	Source	No.
Emerald ash borer	*Agrilus planipennis*	Beetles	FO EAB Biocontrol Facility, MI	40
Asian longhorned beetle	*Anoplophora glabripennis*	Beetles	S&T Otis Lab, MA	20
Coconut rhinoceros beetle	*Oryctes rhinoceros*	Beetles	Univ. HI, Honolulu, HI	20
Khapra beetle	*Trogoderma granarium*	Beetles	S&T Otis Lab, MA	40
Mexican fruit fly	*Anastrepha ludens*	Flies	S&T Mission Lab, TX	40
Oriental fruit fly	*Bactrocera dorsalis*	Flies	ARS, Hilo, HI	40
Mediterranean fruit fly	*Ceratitis capitata*	Flies	ARS, Hilo, HI	10
European cherry fruit fly	*Rhagoletis ceraci*	Flies	Cornell U., NY	55
Asian citrus psyllid	*Diaphorina citri*	True bugs	S&T Mission Lab, TX	40
Spotted lanternfly	*Lycorma delicatula*	True bugs	PA. Dept. Ag.; PA State Univ., PA	55
Red imported fire ant	*Solenopsis invicta*	Wasps, bees, ants	ARS Gainesville, FL	10
Black imported fire ant	*Solenopsis richteri*	Wasps, bees, ants	ARS Gainesville, FL	10
Light brown apple moth	*Epiphyas postvittana*	Moths	S&T Otis-Salinas Lab, CA	40
Old world bollworm	*Helicoverpa armigera*	Moths	S&T Otis Lab, MA	40
European grapevine moth	*Lobesia botrana*	Moths	S&T Otis Lab, MA	40
European gypsy moth	*Lymantria dispar dispar*	Moths	S&T Otis Lab, MA	100
Asian gypsy moth vs. European gypsy moth	*Lymantria dispar asiatica* vs. *L. dispar dispar*	Moths	S&T Otis Lab, MA	25
Japanese gypsy moth vs. European gypsy moth	*Lymantria dispar japonica* vs. *L. dispar dispar*	Moths	S&T Otis Lab, MA	5
False codling moth	*Thaumatotibia leucotreta*	Moths	Citrus Res. Int'l, South Africa	20
Total				**650**

(Table 1).

Riker mounts are portable boxes that display insects under glass (Figure 1) Otis Laboratory coordinated delivery to PDIC of supplies and specimens from nine colonies of invasive insect species maintained at the Otis Insect Containment Facility. We additionally sought out specimens of other species from several U.S. and international sources. Institutions that supplied specimens are detailed in Table 1. Otis Laboratory also created 100 life-cycle displays of European gypsy moth (Figure 1A) and 30 displays of adult European gypsy moth with Asian and Japanese gypsy moth. In addition to preparing specimens for display, PDIC compiled the Riker mounts, produced high-quality magnified images of smaller species for inclusion (e.g., Figure 1B), and acquired cherry fruit fly specimens (Figure 1D).

To reduce the risk of damage to the specimens from carpet beetles and mold, the displays are stored in sealed bins with desiccant packets. In the event that carpet beetle damage is detected, affected Riker mounts can be frozen at -18°C or lower for one month to kill the beetles.

Viewing and requesting Ricker displays from the collection

The lending collection is managed by the FO Outreach Coordinator. It was announced and made available to FO and SPHDs on November 26, 2019 along with a SharePoint link for a photo gallery of displays: Creative Ideas for Your Outreach Events. Additional examples of the Riker displays are shown (Figure 1). Requests to borrow displays should be emailed to camille.e.morris@usda.gov at least two weeks ahead of the event.

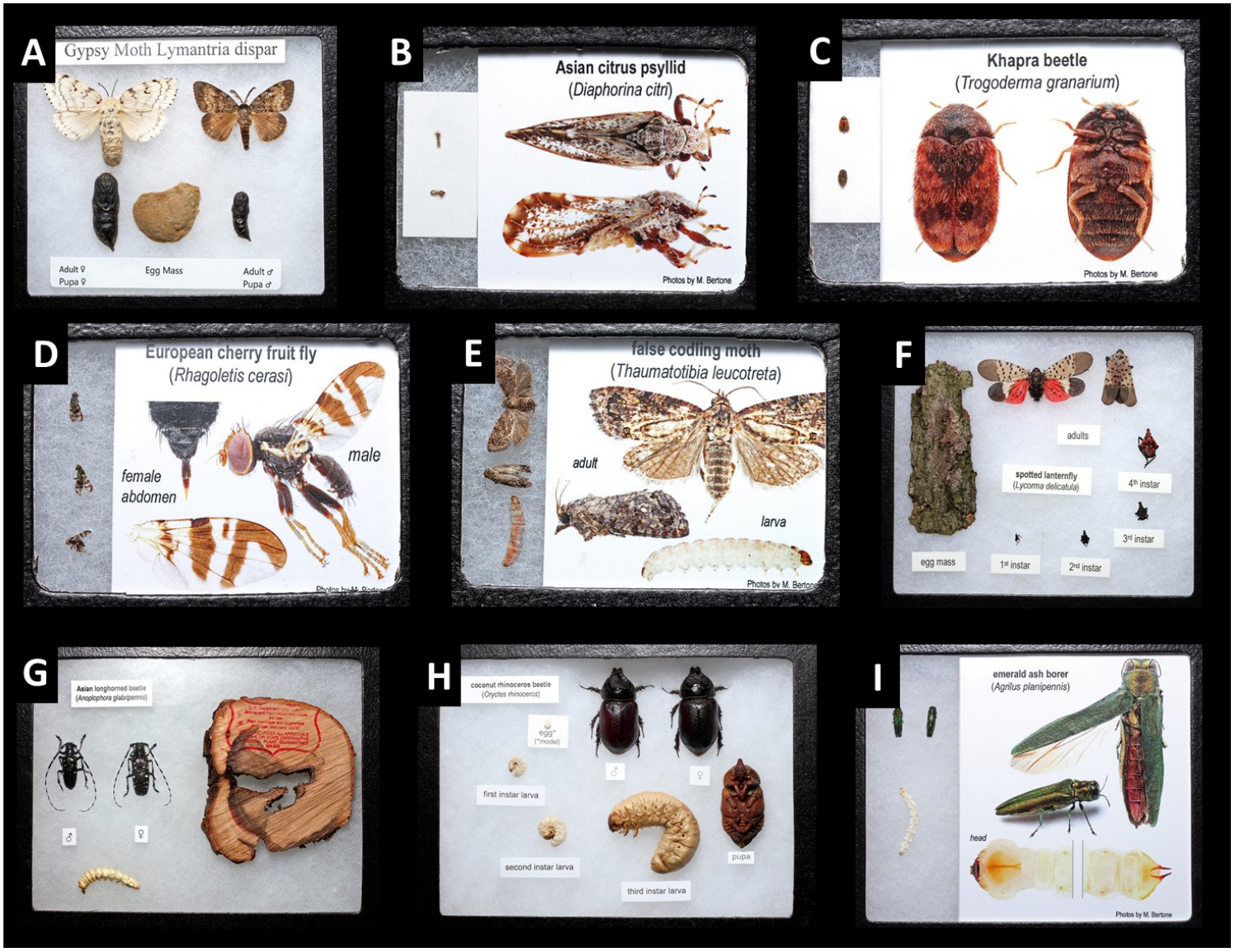

Figure 1. Examples of some of the 19 displays offered by the outreach lending collection including A) life stages of European gypsy moth, B) Asian citrus psyllid, C) khapra beetle, D) European cherry fruit fly, E) false codling moth, F) spotted lanternfly, G) Asian longhorned beetle, H) coconut rhinoceros beetle, and I) emerald ash borer.

Rearing support for the development of a lure for Chinese citrus fruit fly

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Introduction

Chinese citrus fruit fly, CCFF, *Bactrocera minax*, is one of the most serious pests of citrus fruits in China, Bhutan, and northwest India. Eggs are laid in immature fruits from May to June, and larvae develop through summer, dropping in autumn to pupate in soil. Shortly after pupation, development stops and diapause occurs; development continues only after cold temperature triggers the transformation of the pupa into an adult. On December 19, 2018, Otis Laboratory received approximately 2,000 field-collected diapausing pupae of CCFF from China for use in lure development [1]. In order to support CCFF lure development research the Otis rearing team needed to ensure 1) adult flies had a prolonged emergence over several weeks rather than a synchronous burst, and 2) adult flies survived to sexual maturity. Prolonged emergence was required to allow a single researcher to process and test many flies. Successful identification of attractive odors is dependent on the collection and testing of volatile chemicals from both sexually immature and mature flies. A publication indicated that a chill temperature of 10°C for 30 or 60 days would break diapause and enable about 78% and 62% of adults to emerge, respectively, from pupae buried in moist sand [2]. Data from another publication suggested that survival of adults to sexual maturity (commonly 15–20 days) can be achieved by providing them with water and a dry mix of sucrose and either yeast or peptone extract as sources of amino acids [3]. To promote the emergence of adults over a multi-week period and to increase their chances of reaching sexual maturity, we subjected pupae to 10°C for three chill durations and offered the adults four diets. Two of the diets were dry mixes of sucrose and a source of amino acids similar to published diets, while the others represented a new approach for CCFF, where the dry ingredients were embedded in an agar matrix.

Methods

Upon arrival, dead (dark or deformed) pupae were discarded and (apparently) viable pupae were buried in a 1.5 cm layer of moist sand in 15 cm diameter Petri dishes; about 200 pupae were used per dish. Pupae were divided into two equal groups and were chilled separately in two different environmental chambers, both set at a constant 10°C for 36, 50, and 64 days (four dishes in each of the shorter treatments, and two dishes in the 64-day treatment), beginning on December 19, 2018. The Petri dishes were transferred into an environmental chamber at 25±2°C, 60±5% RH, and L:D 15:9 on January 24, February 7, and February 21, 2019. Filtered water was added to the soil as needed throughout the pupal period to maintain moisture. The number and sex of emerged flies were recorded daily and a subset of randomly-selected flies was transferred to cups in same-age and same-sex groups. Up to four flies were placed in a cup and offered one of four diets in a 2.5 cm diameter polyethylene dish. Water was provided by a cotton dental wick protruding from a reservoir below the cup (Figure 1). The effect of diet on adult longevity was compared. Diets consisted of sucrose (table sugar) and either yeast autolysate (Sigma Aldrich, St. Louis, MO, Product 73145) or bovine proteose peptone (Sigma Aldrich, St. Louis, MO, Product 82450) as sources of amino acids. The weight ratio of sucrose to yeast or peptone product was 3:1 in all diets. The agar was GUMPLETE™ Agar (TIC Gums, Belcamp, MD, Product 38760900US).

Figure 1. Diet arenas consisted of a lower cup serving as a water reservoir and an upper cup with a hole in the bottom, placed over the water. A dental wick protruded from the reservoir into the upper cup to serve as a water source. Diets were offered in polyethylene dishes in the upper cups.

Diets:

- DY: Dry sucrose and yeast autolysate
- DP: Dry sucrose and proteose peptone
- AY: Agar block containing sucrose and yeast autolysate
- AP: Agar block containing sucrose and proteose peptone

DY and DP diets were made by weighing and mixing dry ingredients in the desired weight proportions. About 0.75 g of dry diet was offered per cage and replenished weekly. AY and AP diets were made by autoclaving a mixture of 500 ml of water, 82 g of sucrose, and 8 g of agar, allowing it to cool to 45°C in a water bath and stirring in 4.1 g of dry yeast autolysate or proteose peptone. Agar diets were poured into air-tight containers and stored at 4°C for up to one week before use. A block weighing 2.5–3.0 g was placed in a dish and replaced every two to three days.

Daily records were kept of adult emergence from pupae in sand, and of mortality in diet cups. The cause of death was recorded as "unnatural" if it resulted from adhesion to sticky diet, drowning, or damage by handling. Flies dying of unnatural causes or removed for lure research were excluded from longevity analyses for this report. Cups with average fly longevity of $\leq$ 4 days were also excluded, as we assumed death was more likely due to handling than to diet. Longevity analyses treated each cup as a replicate.

Results

A total of 807 flies emerged, of which 392 were male and 415 were female. Percentages of flies emerging after pupal chill in the two environmental chambers were similar (42% and 43%), thus data from both chambers were combined. Percentages of emerged flies and sex ratio were similar across chill durations (Figure 2).

However, the period between end of chill and emergence differed. Adults from pupae exposed to 36 days of chill emerged after a significantly longer period than adults from 50 and 64 days of chill (means ± s.e. were 35.1 ± 0.6, 32.6 ± 0.7, and 31.3 ± 1.1 days, respectively) (ANOVA, F2,123, $p <$ 0.0021, followed by Tukey-Kramer HSD means comparisons, $\alpha = 0.05$). Males tended to emerge one day earlier than females. Overall, the emergence period spanned from February 15 to March 29 (Figure 3).

Applying a conservative estimate of 15–20 days for CCFF adults to reach sexual maturity, 191 flies succeeded: 78 males and 113 females survived 21 days or longer on the provided diets. The numbers of diet replicates suitable for longevity comparisons (cups of flies that died of natural causes) were 24, 21, 25, and 35 for AP, AY, DP, and DY diets, respectively.

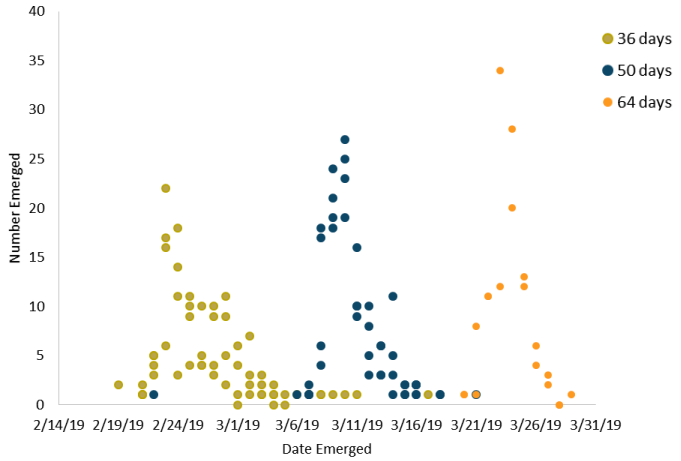

Figure 3. Distribution of fly emergence over time from field-collected pupae exposed to 10°C for three chill durations in the lab.

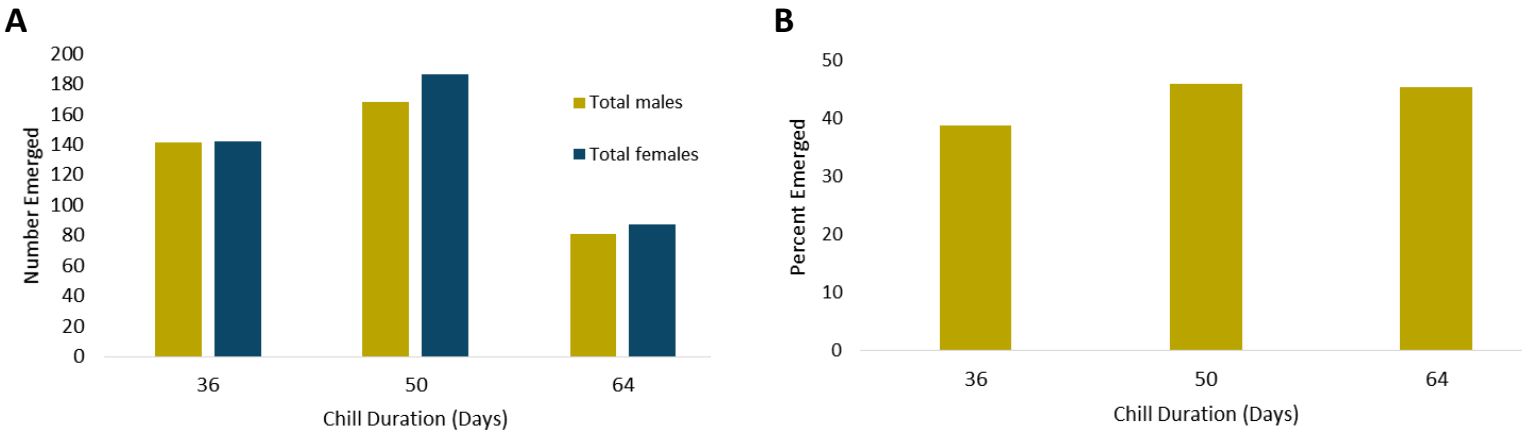

Figure 2. Effect of pupal chill duration at 10°C on A) sex ratio and B) emergence of CCFF.

Regardless of diet presentation as a dry mix or in agar, the four diets did not significantly affect male or female longevity; however, the source of amino acids affected female longevity. Females lived significantly longer when their diets (dry and agar combined) included yeast autolysate than proteose protein (mean ± s.e. 31.9 ± 3.5 and 44.8 ± 3.1 for P and Y diets, respectively; t-test, $p < 0.0081$) (Figure 4). Fly longevity on each diet ranged from a low mean + s.e. of 35.7 ± 4.4 days for DP to a high mean of 46.6 ± 4.2 days for AY. Flies consistently survived equally well or better on the AY diet than on the other diets (Figure 5).

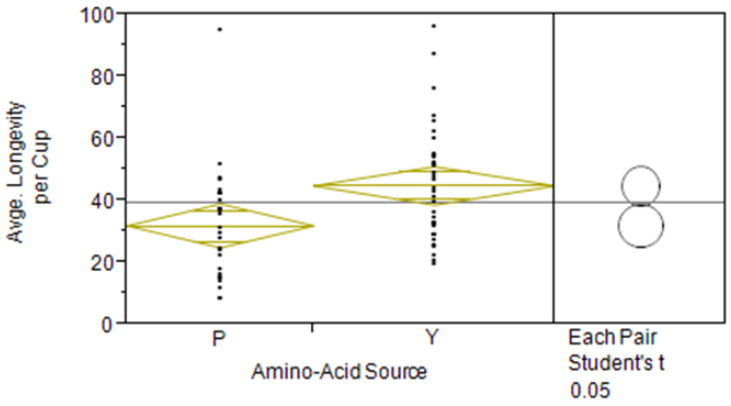

Figure 4. Female CCFF longevity (in days) in response to sources of nitrogen and amino acids. Data points represent mean longevity per cup. P: proteose protein; Y: yeast autolysate.

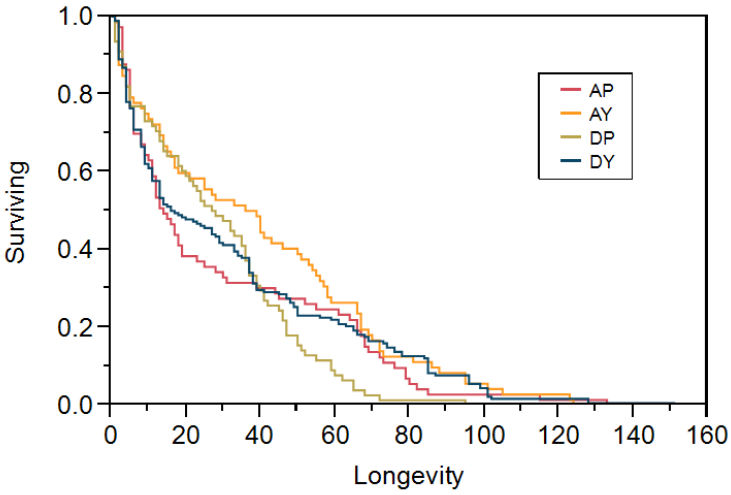

Figure 5. The proportion survival of adult CCFF over time (days) when provisioned with different diets. Lines represent data from all flies per diet.

Conclusions

The first objective of prolonging fly emergence over several weeks was met by subjecting pupae to three chill durations; each treatment resulted in emergence lasting about one to two weeks but combined, the three chill periods resulted in emergence spanning six weeks. Chill duration affects the period between the ending of chill and emergence, with fewer days needed as chill duration is increased. This seemingly unexpected phenomenon has been reported in CCFF [2] and is thought to synchronize fly emergence with seasonal availability of young citrus fruits as hosts. The second objective of producing sexually mature flies in sufficient numbers for lure research was also met by maintaining the flies with water and diets containing sources of energy (sugar) and protein building blocks (amino acids). Both yeast autolysate and proteose peptone are suitable sources of amino acids for maintaining adult CCFF, but females live longer on a diet containing yeast autolysate. The results apply to the development of a mass-rearing system for CCFF.

References

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Longhorned beetle production activities

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Introduction

Five colonies of Asian longhorned beetle, ALB, *Anoplophora glabripennis*, were mass-reared in 2019 in the Otis Insect Containment Facility (OICF). The maintained colonies originated from collections in China, Ohio, Massachusetts, and New York. A genetically mixed colony of beetles from Massachusetts and Ohio is also reared. These colonies supported research and development on the detection, control, and eradication of the pest, and provided specimens for outreach.

Small colonies of two other longhorned beetles from China are also maintained in the OICF: velvet longhorned beetle, VLB, *Trichoferus campestris*, and Chinese mulberry beetle, CMB, *Apriona rugicollis* (formerly know as *A. germari*). Both species have been found in wood packaging material with trade goods from China. Chinese mulberry beetle is not established in North America but has been found in wood packaging arriving in Europe from China.

Asian longhorned beetle

Research programs and public outreach were supplied with specimens from ALB colonies in 2019 (Table 1). Details of supported research projects are detailed here.

Molecular diagnostics: Specimens were provided to cooperators affiliated with Cornell University and the University of Tennessee for the development of diagnostic screening tools.

Rearing methods development: Research continued on a method to rear ALB on natural willow bolts in the OICF. However, pupation remains a challenge because bolts deteriorated as larvae approached maturity. Mature larvae were extracted and held in nutrient-free substrates for pupation, but results were poor.

Chemical ecology: Larvae were reared in bolts of several rooted species of willow and reared adults were utilized in research to identify female pheromones.

Plant resistance: Live eggs and larvae were provided to Purdue University to research the effects of certain plant-defensive phenolic compounds (tannins) on the growth and development of woodborers in multiple tree species. Tannins are being studied in woody tissues, in contrast to earlier research, which focused on leaves.

Outreach and training: Adult and larval specimens were preserved for federal, state, and academic outreach and training displays. Frass produced by larvae reared in willow bolts were provided to the ALB Program in Worcester, MA to train surveyors to recognize ALB infestations.

Velvet longhorned beetle

The VLB colony is currently being used to determine winter chill temperatures required for this insect to complete its life cycle. Closing this knowledge gap will enable us to expand the colony and support ongoing research on the beetle's host range and pest potential. A small-scale study suggested that mature VLB larvae reared on artificial diet pupate at significantly higher rates when exposed to simulated winter at 10°C for three to four months than when maintained in constant 23°C conditions. Diapause in larvae was induced by exposure of mature larvae to fluctuating daily temperatures simulating fall conditions in a temperate climate for one week. In a preliminary trial larvae chilled at 5°C did not pupate; however, these larvae were younger than those in the 10°C treatment group. Increased survival to the adult stage at 10°C resulted in colony growth in 2019 from about 20 to 60 individuals. A second trial, with larger numbers of larvae and randomized ages, is in progress.

Chinese mulberry beetle

The colony was maintained for potential non-target host testing for biological control agents of ALB but was terminated in 2020 due to lack of need. Both CMB and VLB were maintained on an artificial diet developed for ALB.

Table 1. Asian longhorned beetle life stages produced in 2019 and specimens provided to research and outreach.

Purpose	Eggs	Neonates	Older Larvae	Pupae	Adult ♂	Adult ♀
Molecular diagnostics	–	–	27	2	40	40
Rearing methods development	–	–	–	–	18	18
Chemical ecology	–	–	–	–	14	18
Plant resistance	455	100	106	–	–	–
Outreach and training	120	240	188	76	484	435
Colony maintenance	4,816	3,332	2,603	2,122	602	408
Total	5,391	3,672	2,972	2,200	1,158	919

2019 Gypsy moth colony production activities

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The New Jersey strain of European gypsy moth, GM, *Lymantria dispar dispar*, is in its 78th generation in colony at Otis Laboratory. 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 fulfills needs for research, training, education, and outreach.

Four strains of Asian gypsy moth, AGM, *Lymantria dispar asiatica* from 1) China, 2) Korea, 3) Mongolia, and 4) central Russia are also maintained in the Otis Insect Containment Facility. A colony of a separate subspecies from Japan, *Lymantria dispar japonica*, and a related species, rosy moth, *Lymantria mathura* are also reared. Past and future applications for these colonies include research to reduce the risk of AGM entry into the country, develop trap technology and molecular diagnostics for enhanced detection and identification, and to study phenology to map areas at risk of establishment. Additionally, hundreds of specimens are used annually for outreach and training displays.

Production activities

The GM colony served several purposes in 2019 (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 to support the continued maintenance of the colony. Eggs and other GM life stages were distributed to 13 U.S. and four foreign institutions. Over 182,000 male pupae were reared for projects affiliated with the Gypsy Moth Slow the Spread Foundation to research mating disruption and efficacy of mating-disruption products. Substerile GM egg masses, which 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 a location where a trapped AGM had the potential to breed in 2019. Trapped moths in the summer of 2020 might suggest breeding between individuals in both years and may trigger eradication efforts—especially if molecular analysis confirms genetic similarity.

Other egg masses were regularly sent to the USDA Agricultural Research Service Lab in Beltsville, MD for research on GM pathogens. Additionally, regular shipments are sent to universities in Austria and Germany for research on insects that parasitize GM and on GM ecology and their effects on plants.

Otis GM colony eggs were also used by a graduate student at Penn State University to study nuclear polyhedrosis virus (NPV) interactions with the gypsy moth gut microbiome and environmental factors that shape gypsy moth behaviors during NPV infection. Pennsylvania Department of Agriculture used Otis GM colony eggs for biological control research on spotted lanternfly, *Lycorma delicatula*. Lastly, many specimens and life-cycle displays were provided for outreach and training programs in the U.S. In 2019, the AGM colonies were utilized only for outreach and training.

Table 1. The purposes and associated number of gypsy moth, GM, life stages and specimens reared and provided in 2019 to U.S. and foreign institutions.

Purpose	Egg masses	Larvae	Pupae ♂	Pupae ♀	Riker mounts	Specimens
Research: GM-microbial interactions	1,255	~500	–	–	–	–
Research: GM biocontrol	1,120	–	–	–	–	–
Research: GM Slow the Spread Foundation	–	–	182,800	–	–	–
Outreach, training, and education	235	–	100	60	140	100
Research: ecology	151	–	–	–	–	–
Estimate egg-hatch date in AGM-detection site	25	–	–	–	–	–
GM colonies	104	–	–	–	–	–
Spotted lanternfly biological control	200	–	–	–	–	–
Research: unspecified	1,000	–	–	–	–	–
Pesticide assays	12	2,500	–	–	–	–
Total	**4,102**	**~3,000**	**182,900**	**60**	**140**	**100**

Maintaining and developing corn earworm, Old World bollworm, and European grapevine moth colonies

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Three moth colonies were reared in 2019 for research: 1) corn earworm, CEW, *Helicoverpa zea*, 2) Old World bollworm, OWB, *Helicoverpa armigera* (Figure 1), and 3) European grapevine moth, EGVM, *Lobesia botrana*. A previously maintained colony of navel orangeworm, NOW, *Amyelois transitella*, was terminated after February 2019. With the exception of CEW, all colonies were held in the Otis Insect Containment Facility (OICF). Both OWB and CEW are destructive to many important crops including corn, cotton, tomatoes, soybean, and tobacco. CEW is native and widespread throughout the United States. OWB is not established in the continental United States; however, it is established in Puerto Rico, and has been detected in the U.S. in traps baited with CEW pheromone. Because trapping surveys for OWB are hampered by the morphological similarity between adult OWB and CEW and the lack of a specific lure for OWB, colonies of both species are being reared to support the development of molecular diagnostics to distinguish between the two species and their hybrids. We continued to maintain a small colony of EGVM, a pest of grapes eradicated from the U.S. in 2016, to use in studies aiming to reduce reliance on radioactive sources for sterilizing insects, and to provide color-marked males for pest-survey training in California.

Figure 1. Old World bollworm.

Old World bollworm and corn earworm

The OWB colony recovered in 2019 from an earlier decline due to unknown causes. Pathogen tests excluded common disease agents that could cause such declines. Larvae continue to be reared on gypsy moth diet with multiple eggs and young larvae in boxes. Individual third instar larvae are later transferred to multi-cell rearing trays with heat-sealed covers (Figure 2) and reared to the pupa stage. Percentages of larvae surviving from third instar to pupa increased from a low of 30–40% in October 2018 to 70–86% in October 2019, during the colony's 22nd generation at the OICF. Rearing protocols for CEW have not changed since 2017. Pupae were provided in 2019 to establish a colony at the University of Puerto Rico to support collaborative work with the S&T Ft. Collins Lab.

Colorado State University has been conducting molecular research with moths supplied by Otis to 1) better understand the genomes of OWB and CEW, 2) map the three-dimensional chromosome-level assemblies for each species, 3) produce the first fully assembled genome of a hybrid between the two (also at the chromosome level), and 4) compare the hybrid genome to its progenitors to understand how the genome is reorganized in the hybrid and how that might contribute to modified pest traits. Young pupae, less than 24 hours old, were deep-frozen (-40° to -80°C) and shipped on dry ice to a company contracted by the university to perform sequencing. Hybrid offspring from male CEW and female OWB were produced three times from ten group-caging mating attempts. Hybrids from the reverse cross were rare and only produced once after 18 group-caging mating attempts. Researchers at CSU also utilized adults to test a new bulk extraction assay for real-time PCR. Other research that utilized the colony included development and optimization of a rapid OWB egg-detection method for use at ports of entry, conducted at the USDA Agricultural Research Service lab in Stoneville, Mississippi.

European grapevine moth

The EGVM colony has been maintained since 2010. Annually, it provides 200 color-marked males for training pest-survey trappers in California. This easily-maintained colony is being retained to serve as a model for future comparisons between new x-ray technology and traditional radioactive sources for the sterilization of insects for sterile-insect releases.

Figure 2. Old World bollworm pupae ready to be harvested at the Otis Insect Containment Facility. The adhesive paper cover is peeled back from the tray to access the pupae. If needed, the paper is re-sealed over the tray with a heated iron.

An improved non-sticky, circle trunk trap for spotted lanternfly

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Introduction

The spotted lanternfly, SLF, *Lycorma delicatula*, is an invasive, phloem-feeding fulgorid generalist native to China, and was first detected in Pennsylvania in 2014. Before 2019, PPQ's trapping methods for SLF included placing glue-covered sticky bands around trunks of host trees to exploit the insects' behavior of climbing up tree trunks. Glue-covered sticky bands were not an optimal solution for two key reasons. First, the bands are messy and need to be frequently replaced as they become covered in both target and non-target insects and debris. Second, SLF fourth instar nymphs and adults have been observed escaping from sticky bands or avoiding capture altogether. To address the two key issues of sticky bands and increase trap efficacy, a project to design and improve upon commercially available traps was initiated. In 2018, the group developed a new non-sticky, re-usable trap: the circle trunk trap (modified pecan weevil trap), which exploits the behavior of SLF walking up and down the trunk of host trees [1]. The circle trunk trap was transferred to the PPQ SLF program in 2019 because the trap provided substantial improvements in efficiency and overall efficacy. The Behavioral Ecology Survey Technology research group continued to optimize the circle trunk trap's design by comparing the efficacy and usability of collection device variations.

Circle trunk trap: jar vs. bag collection device comparison

An assay was conducted in 2019 at four locations within Trexler Nature Preserve, Lehigh County, Pennsylvania to identify the most effective method of collecting SLF from the circle trunk trap. All circle trunk traps were modified from the preexisting weevil sized opening to accommodate the larger body size of SLF by widening the internal funnel that leads to the collection container. Two variations of collection containers were compared. The first variation consisted of traps fitted with the standard plastic storage jar used in 2018 (Figure 1). The other trapping variation used a gusseted plastic bag (Figure 2) as a collection device. The collection bag variation could be a potential improvement over the jar as it would allow the user to easily replace a full bag with an empty one, and transfer the full bag to the lab or office for sorting. The bag fitted traps were constructed by removing the end of the original pecan weevil trap cup and adding a "tongue" created from acetate sheeting (Figure 3) glued to the inside of the cup.

Figure 1. SLF circle trunk trap design with jar collection device, developed in 2018.

Figure 2. SLF circle trunk trap design with a plastic gusseted bag, developed in 2019.

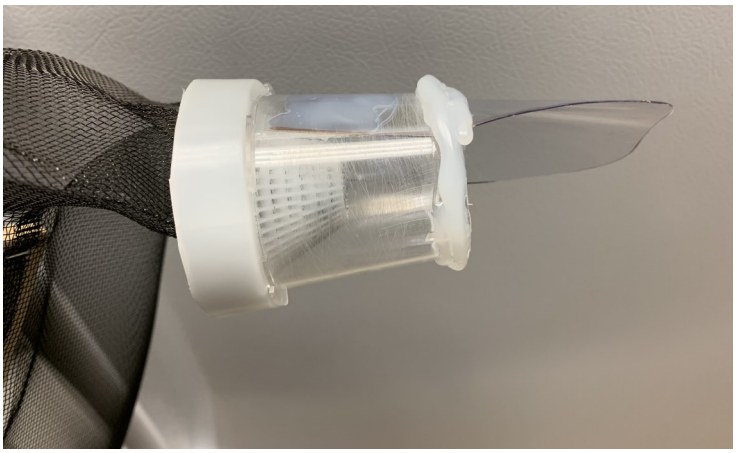

Figure 3. Depicted is the original pecan weevil collection cup modified for the bag collection device. The end of the cup is removed and acetate sheeting is attached to the top of the cup. A line of hot glue is placed around the edge of the cup to provide a synch point for the cable tie.

To compare the two collection containers, traps were placed in a paired study design replicated on 20 pairs of similarly sized *Ailanthus altissma* trees.

Traps were then checked and rotated to the opposite tree in the pair, to reduce tree effects, every three weeks until October 21st. All collected SLF were evaluated for developmental stage and to determine the sex of adults. Over the trapping period, a total of 106,321 individual SLF were caught. Significantly more total nymphs and adults were caught in circle trunk traps fitted with bags than with jars (Figure 4). Additionally, more 1st and 2nd instar nymphs were caught in bags than in jars as well.

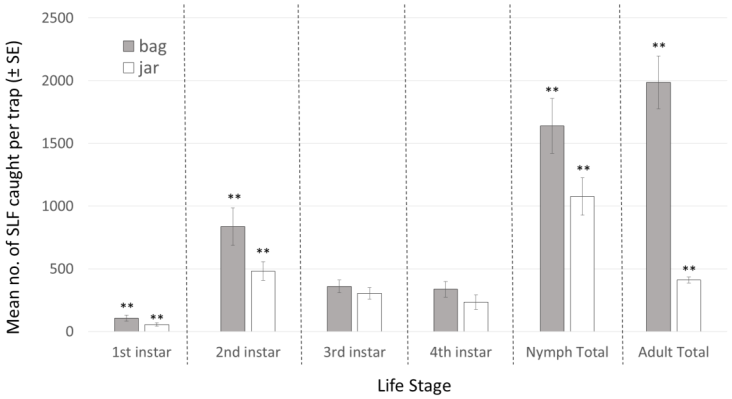

Figure 4. The mean number of SLF nymphs and adults caught in a trapping assay conducted in Lehigh County, Pennsylvania, comparing two versions of the SLF circle trunk trap (n = 20). ** indicates a significant difference in trap catch between treatments within a life stage (paired t-test; $p < 0.05$).

The increased trap catch in the bag over the jar variation may be due to several factors. While the bag is periodically changed throughout the season, the jar is reused and can become covered in honeydew and sooty mold (Figure 5), which may reduce the light entering the jar and interfere with positive phototaxis. The jar must be fastened to the tree to prevent obstruction of the entry port by the body of the trap. However, this positioning can result in the entry becoming obstructed by the accumulation of dead SLF. In contrast, the bag trap is held open by the "tongue," which prevents the bag from closing on itself. The flexible nature of the bag over the rigid jar allows the collected insects to fall into the bag (Figure 6) rather than to obstruct the entry port.

Figure 5. Jar collection device covered in sooty mold following a full trapping season (four months) in the field.

Figure 6. Bag collection device during adult flight during a periodic check. Three weeks had passed since the trap had last been checked.

Conclusions and plans for 2020

In terms of the number of SLF caught, the circle trunk bag variation was an improvement over the jar version. However, the key to a successful survey trap is its ability to detect insects at a very low population density. In 2020, with PPQ Field Operations and state cooperators, we will conduct a field trial comparing detection rates (the ability to catch at least one SLF) for both variations of circle traps along with a BugBarrier tree band in very low-density locations or locations with emerging populations. We will also initiate field trials in Pennsylvania to develop a trap that can be used to trap dispersing adults in flight.

CAPS lure support, specialty lures, and quality control

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Introduction

The Otis Laboratory supplied over 80,000 lures for 32 different insect species to the Cooperative Agricultural Pest Survey (CAPS) program in 2019 (Figure 1). The formulation of these lures starts with obtaining the chemical compounds that make up an insect pheromone. In some cases, this can be a single compound, but more often, it is a blend of several compounds mixed in a very precise ratio that is specific to the insect species. The chemical properties of the pheromone components are very diverse and have different shelf lives.

Specialty lure formulation and quality control

A typical lure formulation procedure starts with ordering the commercially produced pheromone compounds using a specific and detailed Statement of Work (SOW), which is based on the current pheromone literature. Once obtained, these compounds are checked by gas chromatography-coupled-mass spectrometry (GC-MS) for the correct chemical structure and analyzed for purity by a GC-flame ionization detector (GC-FID). The stock of these compounds are checked regularly for stability and shelf life, and are stored at -20°C. Currently, Otis Laboratory has more than 90 different pheromone compounds in stock.

For a multi-component pheromone, the components are mixed according to the necessary ratio and dosage (0.1 – 3.0 mg/septum). At this stage, different stabilizers can be added to the formulation, since some pheromone compounds can quickly degrade when exposed to ambient temperature (>25° C), UV, and/or oxygen. The completed blend is again checked by GC-MS and GC-FID to verify ratio and dosage. Once verified, the pheromone is loaded onto a rubber septa, which allows for a steady release rate over time; additional lure dispensers (e.g., silicone wavers and plastic polymer strings) are also used for lures produced at Otis. The pheromone septa (or other dispenser) are individually sealed in aluminum-coated envelopes and labeled with insect species name, CAPS lure name, manufacture date, and expiration date. Finally, the lures are shipped to the CAPS warehouse (Edinburg, TX) or directly to stakeholders for further distribution.

The Chemical Ecology and CAPS Lure Group regularly examines field-deployed lures by analyzing the remaining active ingredients and degradation to determine the average field longevity for a particular formulation.

When needed, Otis Laboratory also performs quality control analysis of active ingredients by extraction and/or release rate quantification for pre-manufactured commercial lures.

2019 Monitoring support

Additionally, Otis Laboratory Chemical Ecology and CAPS Lure Support Group plays an active role in monitoring the different species of gypsy moths, both domestic and abroad, by supplying specialized lures and conducting active ingredient analysis on mating disruption formulations (Slow the Spread). The group also supports the Forest Service by analyzing new mating disruption formulations for gypsy moth species.

Figure 1. An example of insect pest monitoring with pheromone traps.

2019 Port and Domestic Gypsy Moth Molecular Diagnostics Survey

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Introduction

For 27 years, the Gypsy Moth Molecular Diagnostics Survey has been conducted to screen for the possible introduction of Asian gypsy moth, AGM, *Lymantria dispar asiatica/japonica*, into the United States. The survey includes two core projects: 1) the port survey, which targets suspect AGM specimens found on vessels entering U.S. ports, and 2) the domestic survey, which analyzes specimens found in traps that are deployed by federal and state agencies within and outside of the federal gypsy moth quarantine. All submitted specimens are analyzed using the Standard Diagnostic Assay, which allows AGM to be distinguished from European gypsy moth, EGM, *Lymantria dispar dispar*. If a specimen intercepted at a port or trapped from an area outside of the federal EGM quarantine fails the Standard Diagnostic Assay, DNA barcoding is utilized to make a final determination that is reported to the USDA's National Identification Services.

2019 Port interception results

During the 2019 survey year, a total of 621 specimens were submitted to the Otis Laboratory for molecular identification. The specimens represent 217 interceptions from 21 U.S. ports of entry, from coast to coast. Of the samples submitted, 580 were identified as *Lymantria dispar asiatica/japonica* (Figure 1); 566 were egg masses and 14 were adult females. Additionally, one egg mass intercepted in Houston, Texas, was determined to be *Lymantria umbrosa*—a closely-related species in the AGM complex. Two specimens were identified as EGM and 31 specimens were a species other than a gypsy moth. Utilizing both the Standard Diagnostic Assay and DNA barcoding, determinations were made for 98% of specimens, with only seven specimens that remain unidentified. Of the 31 specimens determined to be a non-gypsy moth, 14 were identified only to genus as the specimens did not match at the species level with any known voucher specimens in the publicly available Barcode of Life Data System (BOLD) database.

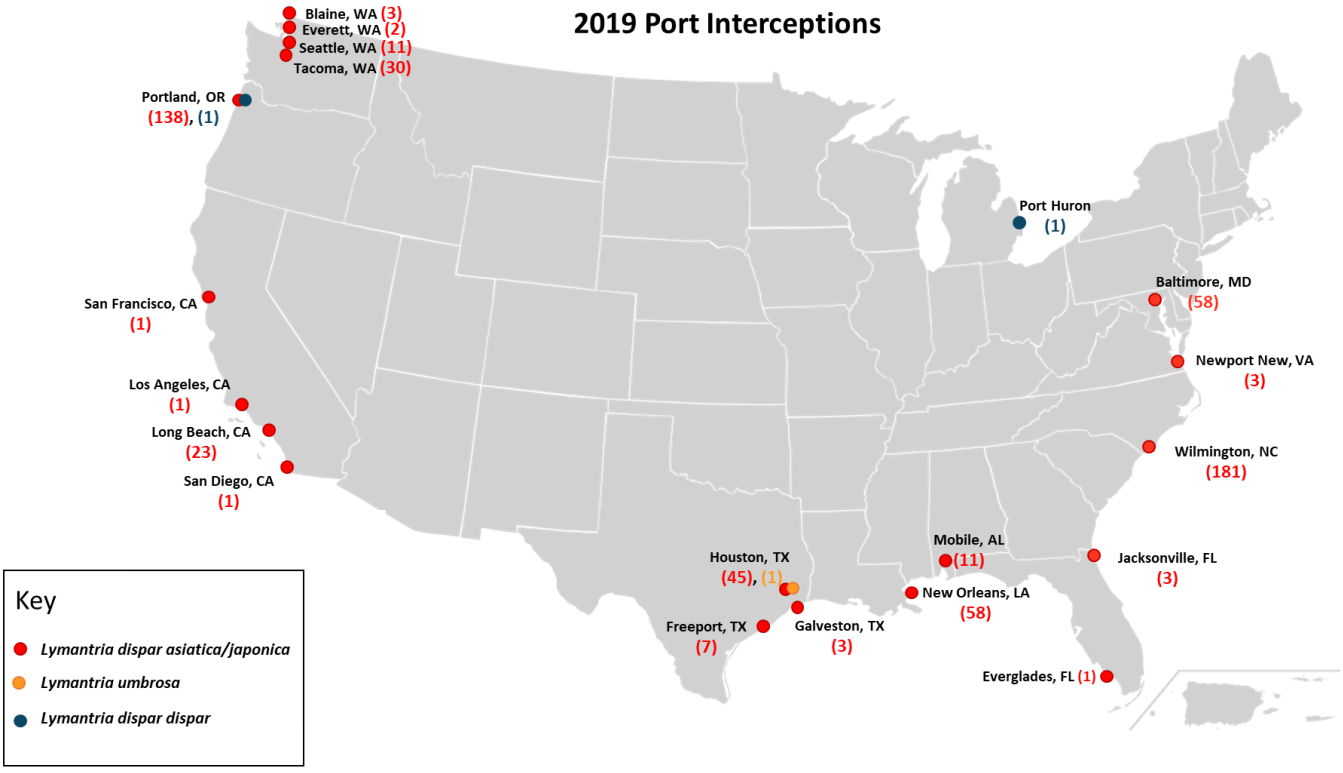

Figure 1. In 2019, 621 specimens collected from U.S. ports of entry were submitted to Otis Laboratory for molecular identification. A total of 580 specimens were identified as *Lymantria dispar asiatica/japonica* at 20 different ports. Red dots on the map indicate the ports where *Lymantria dispar asiatica/japonica* were intercepted, while the number in parenthesis indicates the number of confirmations from each port. Additionally, one specimen intercepted in the port of Houston, TX was identified as *Lymantria umbrosa* (denoted by a yellow dot). Two specimens were determined to be *Lymantria dispar dispar* (one from Portland, OR and one from Port Huron, MI; denoted by blue dots).

The amount of AGM confirmations made in 2019 represents a substantial increase compared to past survey years. Between 2018 and 2019, the number of AGM detected at U.S. ports of entry increased by approximately 630%. Though port AGM detections seem to be on an upward trajectory over the past three survey years, it will be interesting to see if this trend continues in subsequent years and how it relates to the outbreak of native populations in Asia.

2019 Domestic results

In 2019, 2,868 specimens were analyzed as part of the domestic survey. Samples were collected from a total of 28 states—14 states outside the federal EGM (Figure 2) quarantine and 14 states within the quarantine (Figure 3). For all specimens analyzed, 2,357 were determined to be EGM, while 489 remain unknown due to diagnostic failure. Twelve specimens from outside the quarantine were determined to be a species other than gypsy moth. Specimens trapped within the quarantine are not typically submitted for DNA barcoding, therefore unknown specimens could potentially be species other than gypsy moth as well as gypsy moth specimens that were too degraded for successful genetic identification. Additionally, nine specimens were identified as *Lymantria dispar asiatica/japonica*; eight from Franklin County, Pennsylvania and one from Mineral County, Nevada. One adult *Lymantria umbrosa* was also detected in Snohomish County, Washington.

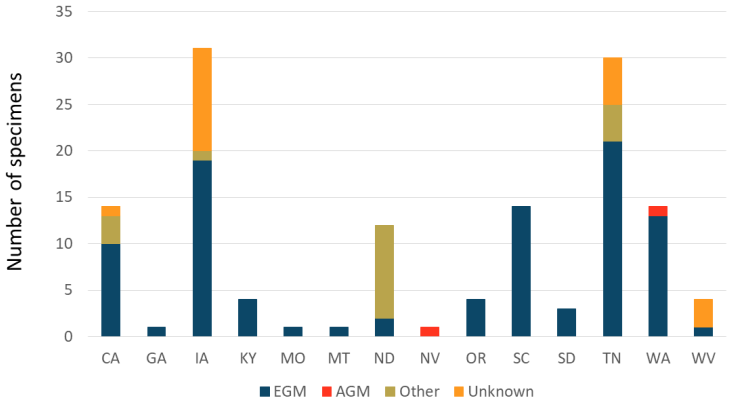

Figure 2. In 2019, 14 states with counties outside of the EGM quarantine submitted a total of 129 specimens for analysis. Ninety four were determined to be *Lymantria dispar dispar*. An egg mass found on a shipping container sent from South Korea to Mineral County, Nevada was determined to be *Lymantria dispar asiatica/japonica*. A single *Lymantria umbrosa* adult was trapped in Woodway, Washington.

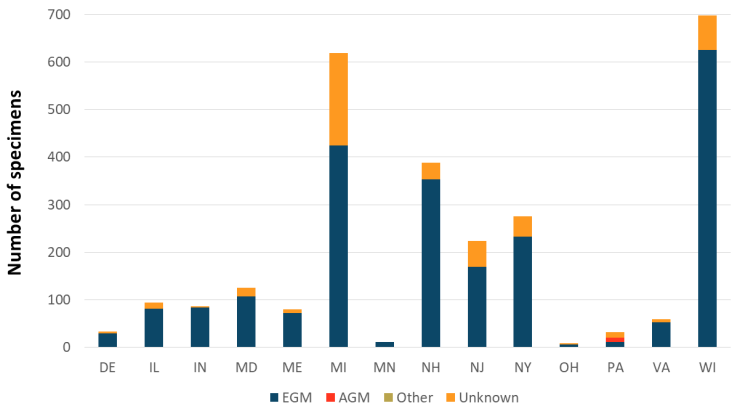

Figure 3. In 2019, 2,739 specimens were analyzed from 14 states with counties that are within the federal EGM quarantine. 2,263 specimens were determined to be *Lymantria dispar dispar*. Eight *Lymantria dispar asiatica/japonica* egg masses were collected in Franklin County, PA.

The nine *Lymantria dispar asaitica/japonica* detections were egg masses discovered by Department of Defense personnel on shipping containers storing military ammunition, which originally arrived on a vessel from South Korea that docked in Concord, California. The containers were subsequently delivered to five army depots, including one in Nevada and one in Pennsylvania. Although the egg masses were collected before hatching could occur in both instances, this serves as a prime example of the pathway by which AGM could potentially be introduced into the U.S. as a result of transportation by maritime vessels.

Conclusion

The importance of the work being done in inspecting vessels at ports of entry by Customs and Border Protection (CBP) personnel and the trapping efforts of PPQ Field Operations is underscored by the results of the 2019 Gypsy Moth Molecular Diagnostic Survey. Efforts to prevent AGM from entering the U.S. by maritime vessel accompanied by additional monitoring were successful in identifying several specimens that could have led to the introduction of AGM into the U.S. The introduction and possible establishment of AGM into the U.S. would result in largescale defoliation, threatening the United States' landscape and natural resources. The Gypsy Moth Molecular Diagnostic Survey, in conjunction with the work done by CBP and PPQ Field Operations, plays an imperative role in preventing and monitoring the establishment of this threatening pest.

Validation of a real-time PCR diagnostic assay for Asian gypsy moth

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Introduction

The current Standard Gypsy Moth Diagnostic Assay functions to distinguish the Asian gypsy moth, AGM, *Lymantria dispar asiatica/japonica*, from the European gypsy moth, EGM, *L. d. dispar*. Recently, Otis Laboratory's Molecular Biology group has been working with the Canadian Forest Service to validate a real-time PCR (RT PCR) diagnostic assay for AGM detection. The new assay allows for the simultaneous identification of *L. d. dispar*, *L. d. asiatica*, *L. d. japonica*, and *L.* umbrosa, which is not possible with the current standard assay. *Lymantria umbrosa*, Hokkaido gypsy moth, is a sister species of *L. dispar* and is a Cooperative Agricultural Pest Survey (CAPS) target pest that was intercepted in the U.S. in 2019.

Methods and results

The RT PCR technique was modified from Stewart et al. [1]. This protocol uses two duplex TaqMan assays (total of four tests) to identify *L. d. dispar*, *L. d. asiatica*, *L. d. japonica* and *L. umbrosa* (Figure 1). An auto-filled species ID tool has been developed in Excel to expedite the interpretation process for diagnostic staff. If the specimen is found to be a non-gypsy moth, DNA barcoding can be employed when necessary.

Table 1. Amplification results for the four tests (1-AGM, 1-Ldaj, and 2-Lda, and 2-Ldd) for the four targeted taxa: *L. d. asiatica, L. d. japonica, L. umbrosa*, and *L. d. dispar*. The positive symbol (+) means positive amplification, and the negative symbol (-) means negative amplification. Amplification curves for each species are via the hyperlinked **appendix 1**.

	1-AGM	1-Ldaj	2-Lda	2-Ldd
Lymantria dispar asiatica	+	+	+	+
Lymantria dispar japonica	+	-	+	+
Lymantria umbrosa	+	-	-	+
Lymantria dispar dispar	-	+	-	+

The RT PCR assay was validated in three steps. First, the level of environmental contamination was evaluated through the range of Ct values (threshold cycle value) for NTC (no template control) samples. Because the molecular laboratory handles thousands of gypsy moth specimens, particles or aerosols containing DNA are inevitably present in the laboratory, despite diligent efforts to control contamination. Results suggested that environmental contamination can produce large Ct values. Therefore, when an unknown sample generates Ct values exceeding those values, the corresponding test should be considered negative.

The next validation step included testing specimens field-collected from known locations. The analyzed specimens included: 25 moths from China, ten moths from the Russian Far East, 53 from South Korea, 77 from Japan, eight from Germany, eight from Italy, and eight *L. umbrosa* from Hokkaido, Japan. Out of the 189 specimens tested, 185 (97.9%) were correctly identified to corresponding subspecies or species. One moth collected in China along with three from Korea, which should have theoretically been *L. d. asiatica*, were instead identified as *L. d. japonica*. It remains unclear whether this inconsistency could have been caused by genetic mutations that led to a false identification. Alternatively, this deviation from the expected result might be explained by the finding of a previous study on gypsy moth population genetics that demonstrated the occurrence of *L. d. japonica* at the southern ports of the Korean Peninsula [2]. Given geographic proximity and trade activity among China, South Korea, and Japan, the introduction of *L. d. japonica* from Japan to mainland Asia and vice versa is certainly plausible.

During the final validation step, we tested 46 eggs from 23 AGM egg masses intercepted between 2014 and 2015 at U.S. ports of entry. The egg masses used had been previously genotyped for next-generation sequencing data and identified as either *L. d. asiatica* or *L. d. japonica*. The RT PCR assay was able to identify all eggs to correct subspecies/species.

Conclusions

We validated a new RT PCR diagnostic assay for AGM that has the potential to increase the molecular tools available for the gypsy moth diagnostic survey. Compared to the current Standard Gypsy Moth Diagnostic Assay, the new assay can simultaneous identify *L. d. dispar*, *L. d. asiatica*, *L. d. japonica*, and *L.* umbrosa, which is not possible with the former standard assay. The adoption of this new technique could boost the specificity and sensitivity of the Gypsy Moth Diagnostics Survey.

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High levels of genetic diversity in invasive *Trichoferus campestris* in the United States

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Introduction

The velvet longhorned beetle, VLB, *Trichoferus campestris*, is an invasive species in North America that is native to Asia. It was first officially recorded in Quebec, Canada in 2002 and again in 2006 [1]. This wood-boring pest has been intercepted in solid wood packaging materials at U.S. ports of entry and during domestic CAPS surveys since 2009. Even though *T. campestris* may pose a threat to many ecologically and economically important tree species, there have been no genetic studies of this pest. In fact, no molecular phylogenetic studies within the genus or the tribe have ever been conducted. Published COI sequences from *T. campestris* revealed considerable intraspecific divergence, which may suggest the presence of morphologically cryptic species or molecular operational taxonomic units (mOTUs, distance/similarity-based specimen clusters) [2]. Here we generated five mitochondrial markers beyond the "universal" COI barcode for *T. campestris* and explored mOTU delimitation for evidence of cryptic lineages. Deciphering genetic diversity of the invasive population can help PPQ tailor lineage-specific responses.

Methods and results

To amplify additional mitochondrial fragments beyond the 658 bp COI barcode, we designed five species-specific pairs of PCR primers for the following genes: a partial fragment of the cytochrome c oxidase subunit II (COII) gene, partial cytochrome c oxidase subunit III (COIII) gene, partial cytochrome b (Cytb) gene, partial NADH dehydrogenase subunit 2 (ND2) gene, and partial NADH dehydrogenase subunit 4 (ND4) gene. With the COI barcode, the complete dataset (3,709 bp) included a total of six mitochondrial protein-coding genes for each specimen.

Analyzed samples included 66 VLB specimens collected from nine U.S. states and 51 specimens intercepted at five U.S. ports of entry. Additionally, four VLB specimens were obtained from the species' native range in China. In the phylogenetic tree (Figure 1), three well-differentiated major mitochondrial lineages can be identified with high statistical support. In each lineage, domestically collected samples mixed with those intercepted at ports.

Specimens collected in Utah did not form a single genetic group but instead were represented by five distinct mitochondrial haplotypes (Figure 1). The haplotypes seemed to be restricted to Utah and were not detected in other states, except for one haplotype that was found in an Ohio specimen. Many domestic sites seemingly hosted a high level of local genetic diversity, as beetles from the same location were placed into multiple mitochondrial lineages. This phenomenon was observed repeatedly across the gene tree regardless of sample size per location.

Pairwise uncorrected *p*-distance among specimens was larger than within-species variation observed in other pest insects. The distance-based delimitation method consistently defined eight mOTUs in the COI dataset. Mitochondrial Lineage I and Lineage II approximately corresponded to two of the eight mOTUs, whereas Lineage III was partitioned into four additional mOTUs. Bayesian analysis estimated many more mOTUs using the complete dataset with numbers ranging between 24 and 61 (mean 41.35). The null model of considering all samples as conspecific (belonging to the same species) was rejected by a likelihood ratio test ($p = 0.003$).

Conclusion

We identified three well-supported mitochondrial lineages, which each contain a mix of samples from domestic locations and port interceptions. Species delimitation analyses suggest multiple mOTUs in *T. campestris* that warrant further taxonomic assessment. Future nuclear data generated through next-generation sequencing will help confirm or contrast the observed mitochondrial diversity as well as the status of those mitochondrial lineages. The study provides critical information on the characteristics of the introduction of *T. campestris,* enabling PPQ to deliver linage-specific responses. A manuscript on these findings was recently published in Biological Invasions [3].

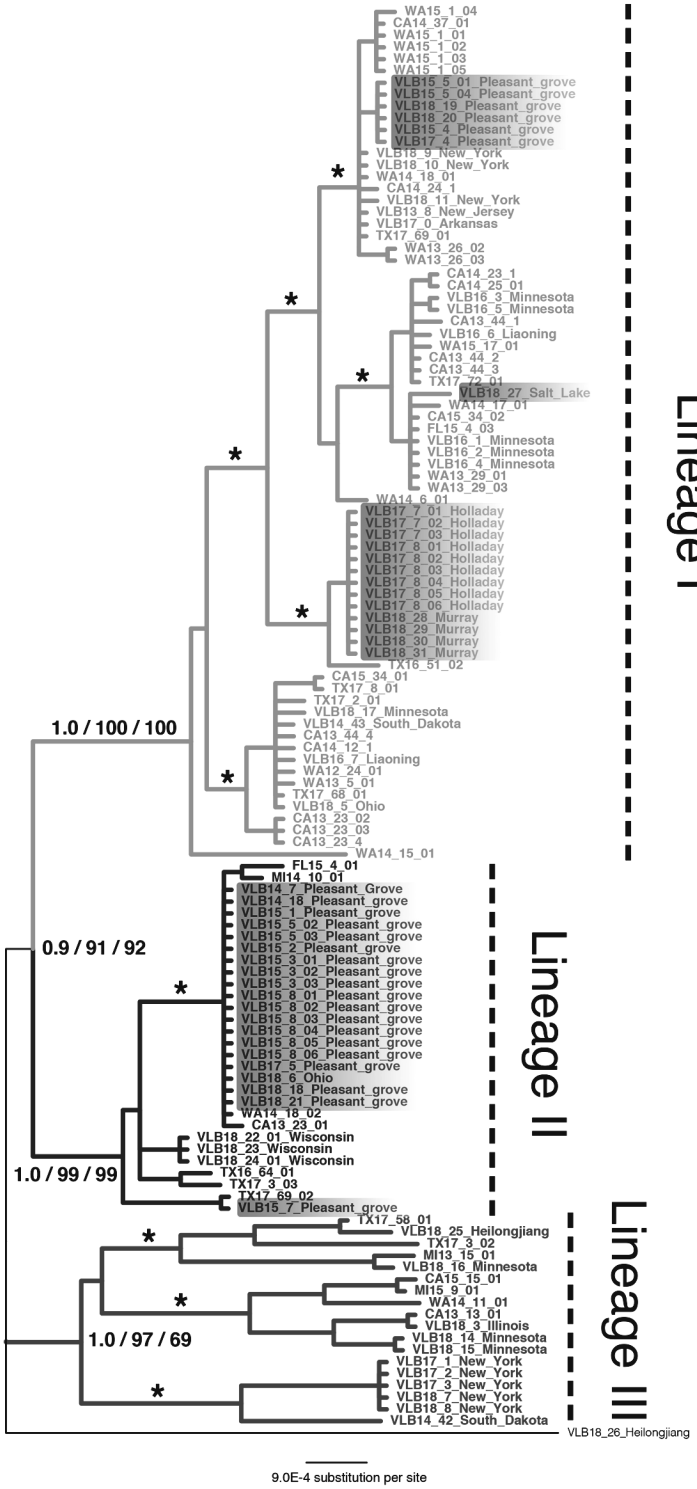

Figure 1. Bayesian mitochondrial gene tree based on the complete dataset. Asterisks indicate high statistical branch support. Specimens from Utah are shaded.

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Genetic structure of Asian longhorned beetle in the United States and China

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Introduction

The Asian longhorned beetle, ALB, *Anoplophora glabripennis*, has three extant U.S. populations: Ohio (Bethel), New York (Long Island), and Massachusetts (Worcester). Characterizing genetic structure among those populations helps explain the process of introduction. In previous years' work, we have generated genome-wide microsatellite data and SNP (Single Nucleotide Polymorphisms) data, which offered new insights into the genetic relationships between extant and eradicated populations. By combining data generated from the beetle's native range in Asia, our work also provides the basis to assign individual ALB to its source population(s). This helps PPQ to track the geographic origin of the invasive population.

Methods and results

In addition to the 176 specimens collected from the three extant populations, we obtained 48 ALB tissue samples from the two infestations in Canada (Ontario and Mississauga), which helped to complete the invasion history of ALB in North America. We also recovered specimens from eradicated populations from Massachusetts (Boston), New York (Staten Island and Islip), and Illinois (Chicago). Over 200 beetles from 16 locations in China were also acquired. All specimens were genotyped for 53 microsatellite loci and 999 SNP loci.

Discriminant Analysis of Principal Component (DAPC) found relatively equal support for models that defined 6–10 genetic clusters among North American populations. A scatter plot showing nine genetic clusters revealed significant geographic patterns (Figure 1). The three extant U.S. populations were confirmed to be divergent from each other. Although previous results suggested a close relationship between the Ohio population and Canadian populations based on mitochondrial DNA, this new nuclear dataset suggests that the two groups are sufficiently different.

Interestingly, divergence within New York and Canada contrasted with previous theories that minimum genetic variation existed within either location. To further assess the variation, samples from New York and Canada were analyzed through DAPC.

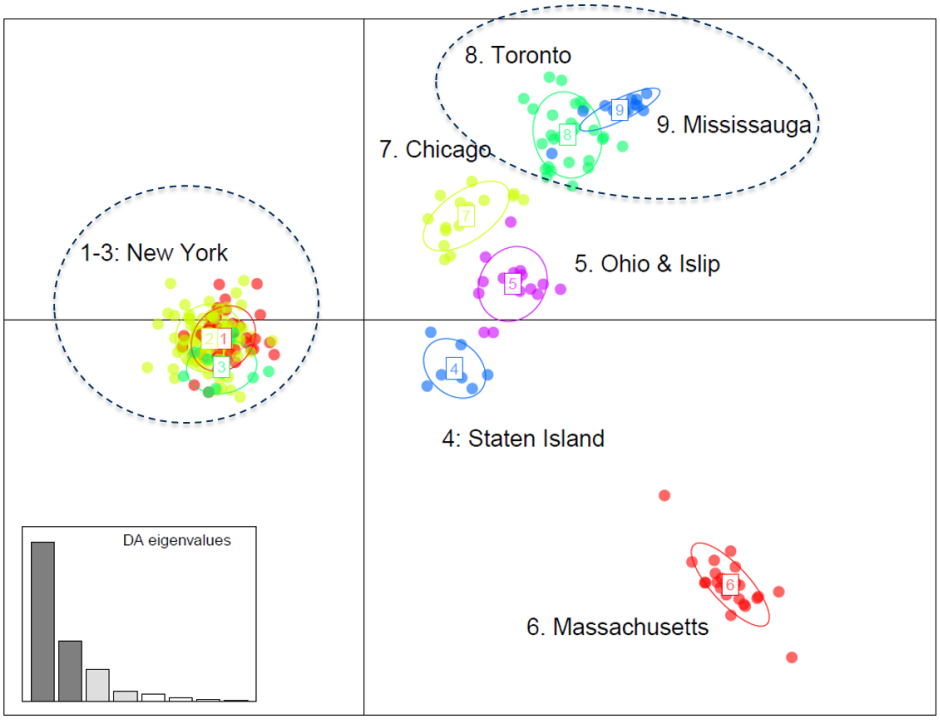

Figure 1. DAPC scatterplot for North American populations under the model of nine genetic clusters. Each dot represents a single specimen. The Massachusetts cluster refers to the Worcester site and the eradicated Boston site. The three New York clusters refer to the three collection sites (Amityville, Farmingdale, and Copiague) on Long Island.

Results showed that although the three New York sites are only a few miles from each other, there is indeed some extent of divergence between Copiague and the other two sites (Farmingdale and Amityville) (Figure 2). Similarly, genetic divergence can be found between two Canadian sites when analyzed alone.

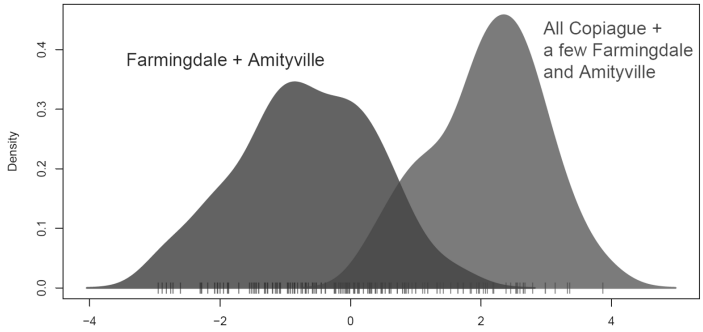

Figure 2. DAPC histogram of the first discriminant function for the three sites on Long Island, New York.

DAPC for Chinese samples showed that there were 4–6 genetic clusters among collected samples (Figure 3). The largest group was formed mostly by samples from the North China Plain, a highly industrialized and populated area that includes Beijing. Two populations from northwest China (Northwest China A and B), which are merely 200 kilometers away, were very distinct in their genetic makeup, with one related to the North China Plain cluster. This could provide evidence of human-aided movement of ALB within its native range.

Assignment tests were performed to predict the geographic origin of genetic clusters in North America. All North American specimens, except for one beetle from Amityville, were assigned the North China Plain cluster. Therefore, our result suggests that this geographic area serves as the major source of introduction in North America.

Conclusions

Newly generated genomic data demonstrated the distinctiveness of the three extant U.S. populations and thus supports the theory of independent introductions. However, most, if not all, North American ALB likely originated from the North China Plain. The results provide important information about the characteristics of the ALB introduction and its connection to source populations in China.

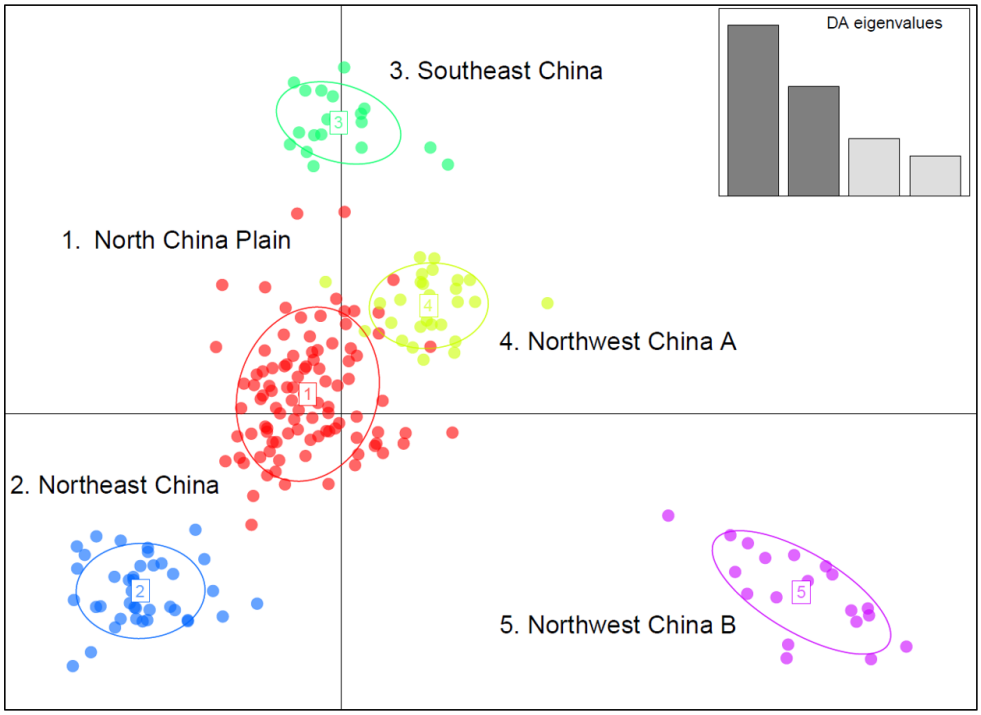

Figure 3. DAPC scatterplot for Chinese populations under the model of five genetic clusters.

Identification of female produced pheromone components of Asian longhorned beetle and the effect on behavior

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Introduction

The Asian longhorned beetle, ALB, *Anoplophora glabripennis,* is a serious pest of over 43 species of hardwood trees in North America, China, and Europe. Estimates of potential U.S. economic damage by ALB, if left unchecked, are in the billions of U.S. dollars. Eradication programs still rely on visual identification of the beetles and their damage to find infested trees and delimit ALB populations. ALB traps and lures currently used need to be improved before they can become an operational tool for ALB survey programs. The development of an effective lure and trap for monitoring ALB has been hindered by the fact that mate finding involves a rather complex series of behaviors and responses to several chemical and visual cues. First, adults locate a tree via host kairomones and then males produce a pheromone to attract other adults. In the final step of mate recognition, males use female-produced trail and contact pheromones. Further research by our collaborators has shown that female ALB contact pheromone components are a potential precursor that undergo abiotic oxidation to yield volatile pheromone components. Our latest research has aimed to identify female-produced pheromone components from ozone-treated ALB body washes and test them for behavioral activity using laboratory and field assays.

Oxidation and analysis of female extracts

A concentrated hexane wash of virgin ALB females (n = 24) was oxidized by bubbling ozone through the extract for five minutes at 0°C. A slow stream of argon was then bubbled through the solution for approximately two minutes and a drop of dimethyl sulfide was added to reduce the ozonides. Volatile compounds derived from this oxidation process were screened for antennal activity using gas chromatography electroantennographic detection and identified gas-chromatography mass spectrometry. Antennally active compounds were then tested for behavioral activity in laboratory olfactometer assays and field trapping tests in China.

Results

Ozone treatments of virgin female extracts yielded sixteen aldehydes, nine of which were found in trace amounts. All sixteen aldehydes elicited antennal responses in both male and female antennae, although responses were clearer and more distinct with standards of 6:ALD, 7:ALD, 8:ALD, 9:ALD, 10:ALD, 11:ALD and 12:ALD. Olfactometer assays showed that males were highly attracted to a blend of these seven aldehydes (7xALD). Females did not show any attraction to the 7xALD blend. Despite low population levels in Chinese field tests in 2018, traps containing the 7xALD blend detected ALB every week over six weeks and caught significantly more adults (mainly males) than empty control cross-vane traps (Figure 1). In the 2019 China field test of the 7xALD and 3 component host blend lure, both caught significantly more males—nearly five times more—than blank control traps (data not shown). Our findings demonstrate the potential for traps baited with both female-produced pheromones and plant volatiles to detecting infestations of *A. glabripennis*.

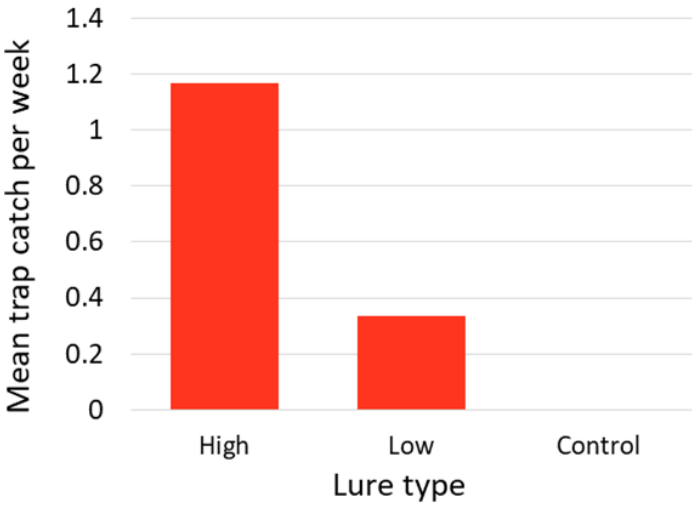

Figure 1. Trap catches of adult ALB to high (30 mg per day) and low (3 mg per day) release rates of a 7 component aldehyde lure from a 2018 China filed study, n = 15.

Preliminary study on rearing and storage of *Diomus pumilio*

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Citrus trees are commonly grown in urban backyards of southern California. Populations of Asian citrus psyllid, ACP, *Diaphorina citri*, on these trees have been recognized as a major source for the continuous re-infestation of commercial citrus orchards in nearby areas. One of the approaches to minimize such migration of ACP is to attempt area-wide suppression of ACP in urban areas. A study is underway in collaboration with Hemet Valley Pest Control District to evaluate the impacts of ant suppression and the release of biological control agents such as *Tamarixia radiata*, *Chrysoperla comanche*, *Rhyzobius lophanthae*, and *Diomus pumilio*.

Diomus pumilio is primarily a predator of psyllid eggs and early instar nymphs. However, it is not currently reared by commercial insectaries. Very little is known about the biology and behavior of *D. pumilio*, which was introduced in California to control Albizia psyllid in the 1970s and successfully established, providing effective suppression of the target psyllid [1]. Renewed interest in *D. pumilio* is due to the recent discovery of its association with ACP [2]. While *D. pumilio* has the potential to help reduce ACP populations, little is known about the beetle's biology under natural or laboratory conditions.

Preliminary studies were conducted to explore the suitability of various psyllid species (ACP, potato psyllid, Acacia psyllid, and Mesquite psyllid) for the rearing of *D. pumilio* in the greenhouse. *Diomus pumilio* successfully developed on all four species of psyllid tested; however, the best results were obtained when reared on Acacia psyllids. Mesquite plants did not produce new flush during winter testing, while *Acacia farnesiana* flushed continuously, even during winter months, supporting the availability of large psyllid colonies for *D. pumilio* rearing. Additionally, unlike ACP and potato psyllid, Acacia psyllid is not a pest of economic importance, therefore, special precautions would not have to be taken to prevent their escape during the rearing process.

Subsequently, ten *D. pumilio* production cages were set up in the greenhouse. Four acacia plants in each cage were inoculated with 400 psyllids and 20 *D. pumilio* adults. On average, the cages produced a little over 400 *D. pumilio*.

Another study was set up in the laboratory to evaluate the effects of various food sources on *D. pumilio* longevity in storage at 64°F. The following treatments were evaluated:

- Blue paper towel (one square inch) soaked in 75% honey solution and excess honey removed
- Frozen *Ephestia* eggs added on top of the honey-soaked paper towel
- Brine shrimp eggs added on top of the honey-soaked paper towel
- No food, only water (control)

Ten *D. pumilio* adults were stored in a 40-dram vial. The number of surviving beetles was recorded at weekly intervals.

Diomus pumilio adults provided with *Ephestia* eggs in addition to honey had the highest longevity (130 days), which was significantly better than the rest of the treatments tested (Table 1). *Diomus pumilio* longevity with honey alone (102.5 days) or with brine shrimp eggs (112.7 days) did not differ from each other; however, both were significantly better than the control (10.7 days). Developing a successful, low-cost, mass rearing method for *D. pumilio* requires the efficient production of beetles and the ability to store beetles until they can be released in the field. With basic production methods in hand, we can now modify our rearing methods to maximize *D. pumilio* production.

Table 1. The longevity of *Diomus pumilio* adults in storage with various food sources.

Treatment	Longevity (days)
Control (water)	10.7a
Honey	102.5b
Honey with shrimp egg	112.7b
Honey with *Ephestia* eggs	130.4c
	$F_{3,12} = 281$ ($p < 0.001$)

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1) Pinnock D, Hagen K, Cassidy D, Brand R, Milstead J, Tasson R. Integrated pest management in highway landscapes. California Agriculture. 1978 Feb 1;32(2):33-6.

2) Goldmann, A. Predators of Asian Citrus Psyllid (*Diaphorina citri*) in Southern California. (Doctoral Dissertation, University of California Riverside) 2017.

Updated *Tamarixia radiata* field cage production: 1 million wasps in 2019

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In 2013 the Salinas Field Station and the Citrus Research Board (CRB) began developing methods for the mass production of *Tamarixia radiata* using large field cages. The wasp production is part of a collaborative Asian citrus psyllid, ACP, *Diaphorina citri*, classical biological control program in Southern California. As *T. radiata* production has steadily increased on an annual basis, rearing methods have been refined based on the outcome of field cage production. However, in 2019 only 1,078,338 wasps were produced, down from the 1,219,037 produced in 2018 (Table 1). While yearly production was slightly down, over 1 million wasps were still provided to the California Department of Food and Agriculture (CDFA) for releases in Southern California. Remaining wasps were used as starter material for field cages. Overall, the collaborative biological control program produced over 4.7 million *T. radiata* in 2019, 23% of which were produced by the CRB program (Table 1).

In 2019, all field cages were set up in an experimental plot at Cal Poly Pomona, beginning in April and continuing through October. A total of 41 cages were established for *T. radiata* production with four of those left up over the winter. Overwintering cages should allow for an easier start to the production season, assuming a high population of psyllid nymphs successfully overwinter. Due to lower spring temperature, *T. radiata* production did not occur until the first week of July (on a cage set up in May 2019). To avoid deleterious impacts on tree health, cages were harvested only once during the season. Cages produced an average of 30,810 wasps in 2019, down from 37,873 in 2018 (Figure 1). However, under optimal conditions, a single cage was able to produce 120,950 wasps (Table 2), an increase of over 30,000 from 2018's highest producing cage. Production of *T. radiata* varied seasonally, with peak production coinciding with the hottest months (Figure 2).

In 2019 nine different host plant varieties were used for *T. radiata* production (Figure 3). Lisbon Lemon and curry leaf each accounted for 19% of wasp production. Lisbon lemon, which was used as a host for *T. radiata* inoculation rate experiments, had the most cages (nine) set up in 2019. The development of new methods for handling curry leaf as a host plant is allowing for greater *T. radiata* production. In previous years curry leaf was pruned down to woody tissue, inoculated with ACP, repruned after ACP maturation, and then inoculated with *T. radiata* on the second generation of ACP. In 2019, curry leaf was inoculated with a higher rate of ACP, allowing *T. radiata* to establish on the first generation of ACP, significantly reducing the number of days before harvest.

Over the years we have made great strides in learning how to efficiently mass-produce *T. radiata* in field cage insectaries and will continue to improve upon these methods.

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.

Agency	2013	2014	2015	2016	2017	2018	2019	Total
CRB	119,142	240,456	510,897	607,019	878,827	1,219,037	1,078,338	4,653,716
FAR	–	137,524	265,961	147,850	205,383	–	–	756,718
UCR	161,057	296,881	165,445	523,015	650,748	400,212	174,310	2,371,668
CDFA	60,626	963,373	1,355,240	990,290	2,045,350	2,562,479	3,458,745	11,436,103
Total	340,825	1,638,234	2,297,543	2,268,174	3,780,308	4,181,728	4,711,393	19,218,205

Table 2. Yearly *Tamarixia radiata* production in field cages.

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	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	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
2019	41	39	30,810 (3,927)	120,950	20.6 (2.7)	73	1,078,338

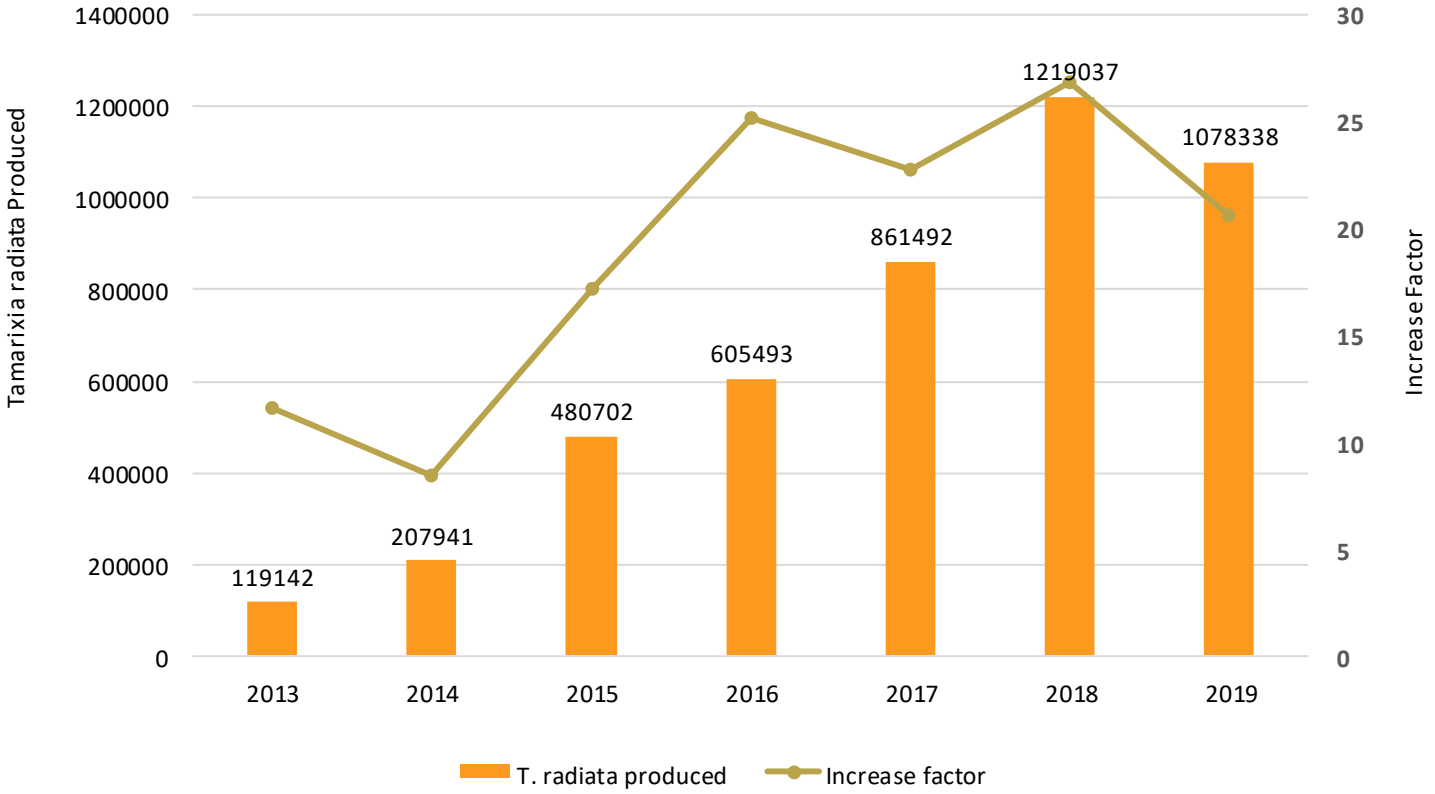

Figure 1. Yearly total *T. radiata* production (columns) and average increase factor (line) in field cage insectaries.

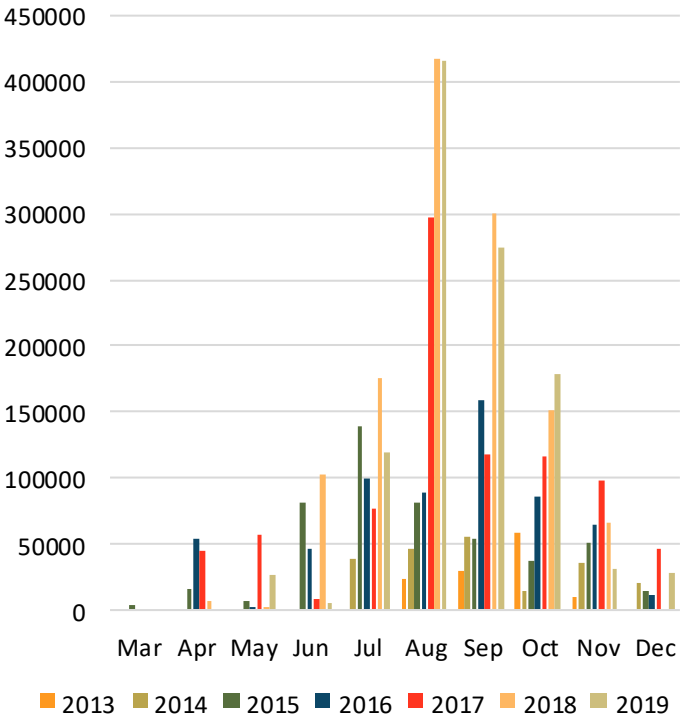

Figure 2. Monthly production of *T. radiata* from field cages.

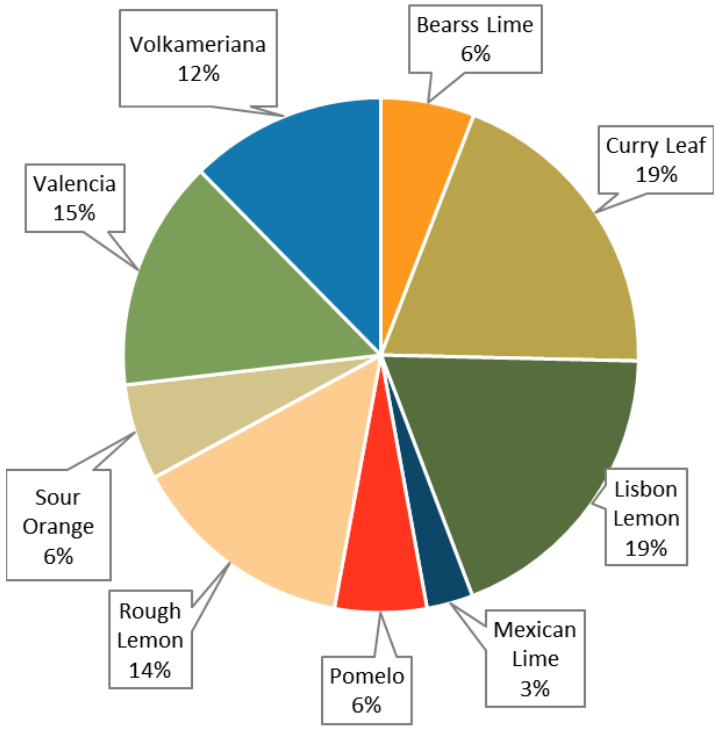

Figure 3. Breakdown of 2019 *T. radiata* production by host plant variety.

Update on classical biological control of Asian citrus psyllid in Arizona using the parasitoid *Tamarixia radiata*

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Introduction

In 2013, efforts began to establish *Tamarixia radiata*, a parasitoid of the Asian citrus psyllid, ACP, *Diaphorina citri*, as an agent of classical biological control. For the past four years, releases have been focused in and around Yuma and Lake Havasu City, Arizona. During 2016 surveys, *T. radiata* was recovered at 23 of 31 survey sites. However, questions remain as to whether or not *T. radiata* has become established, especially as ACP populations decline to undetectable levels during mid-summer in the Yuma area [1]. Releases in Yuma and Lake Havasu have ended, and monitoring will continue in Yuma in the absence of releases to evaluate *T. radiata* establishment.

The focus has now shifted to four new areas (San Luis, Wellton, Ajo, and Nogales) with established ACP populations where *T. radiata* releases will be made and ACP and *T. radiata* populations monitored. Biocontrol agents continue to be supplied from the iso-line colonies reared at the University of California, Riverside (UCR). Releases and monitoring are conducted by USDA APHIS PPQ Field Operations staff in Arizona.

New regions

Two of the new release and monitoring regions in Arizona, San Luis, and Wellton are near Yuma and share a similar climate. Ajo is at a higher elevation and undergoes less extreme high temperatures in the summer months than Yuma. Nogales has a semi-arid climate and cooler temperatures than either Yuma or Ajo. While seasonal ACP population fluctuations are likely to show similar patterns in San Luis and Wellton as they have in Yuma, the less extreme summer heat in Ajo and Nogales may allow ACP to remain at higher population densities for longer periods without a dramatic drop in numbers during mid to late-summer (Figure 1).

Releases of *T. radiata* have previously been made in San Luis, but continue now at new release/survey sites within the region and with a formal release and monitoring protocol put into place. *Tamarixia radiata* has previously been detected in Wellton, despite the nearest release site at the time being 23 km away.

Releases of *T. radiata* were previously made in Ajo in 2018 when high levels of ACP infestation were observed in this area. A program of regular surveys and *T. radiata* releases has now been put into place in Ajo. Six release/monitoring sites have been established in this location so far. Nogales pre-release surveys in this site showed heavy ACP infestation in residential sites, along with evidence of parasitism by *T. radiata* at several of these sites despite no USDA releases of the parasitoid having been made there in the past.

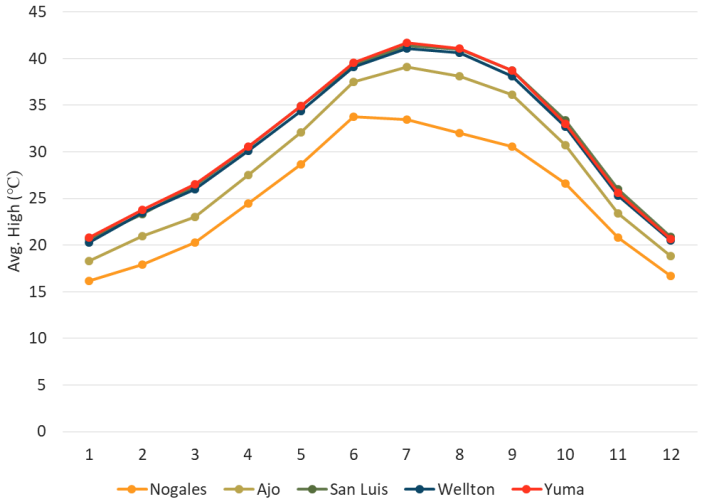

Figure 1. Monthly average high temperature in Yuma, AZ, and the four new classical biological control regions. Data collected from https://en.climate-data.org/

Release and monitoring protocol

After meeting with cooperators in Arizona, a new, unified protocol was developed for site selection, *T. radiata* release, and ACP and *T. radiata* monitoring for all biological control regions. Each region receives one shipment of 2,000 *T. radiata* per month. Releases and surveys take place at the same sites, with surveys taking place either one day or one week before *T. radiata* release to prevent recently released *T. radiata* from being counted. Releases are made at each site once every four weeks. The numbers of adult ACP and *T. radiata* are monitored by yellow sticky cards that are placed in one tree per site to be collected and changed monthly.

Adult ACP are also monitored by tap sampling, with a sample taken on each side (North, East, South, and West) of at least one tree per site. Two branches on each side of the tree are then randomly selected and the stages of flush on the branch are identified and recorded. The presence or absence of ACP

eggs and nymphs and ants tending psyllids are also noted on sample branches. If citrus flush with immature ACP are numerous, up to two shoot samples are collected and frozen for two days before being shipped overnight in insulated containers to the Otis Lab Salinas Field Station. A dissecting microscope is used to count ACP eggs, 1st through 3rd instar nymphs, and 4th through 5th instar nymphs. Nymphs are then flipped over to look for *T. radiata* eggs and larvae underneath. Intact mummies and mummies with exit holes are also noted. These counts are used to estimate percent parasitism.

When *T. radiata* was found at several Nogales sites prior to USDA releases, samples were taken to attempt to determine their origin. Approximately 30 adult *T. radiata* were collected from two heavily ACP-infested sites and placed in alcohol; samples were sent to UCR for genetic analysis.

Results

Releases of 2,000 *T. radiata* have been carried out monthly in all-new release areas. Monitoring data was collected for October, November, and December 2019 in all regions except for Ajo, where monitoring data were not collected in December.

The first counts of ACP adults at surveys sites in Nogales and Ajo, conducted in October, were done as timed visual counts of adult ACP on trees, with surveyors counting for two minutes per tree quadrant. For subsequent surveys, the sampling method was changed to tap sampling to match similar surveys being conducted as part of an area-wide integrated pest management project taking place in Hemet, CA. Because of this change in sampling methods, comparison of ACP adult counts in October to those in later months is difficult; however similar numbers of ACP were seen in Ajo and Nogales in October and again in November (Table 1). The number of adult ACP in tap samples decreased from November to December in Ajo. The percent of sampled branches with ACP eggs or nymphs present declined from October to November in Ajo but increased through December in Nogales (Table 2). No ACP adults, nymphs, or eggs were detected in Yuma, Wellton, or San Luis during recent surveys.

One set of shoot samples, consisting of three shoots collected in late October from three separate sites, was shipped from Ajo and examined. ACP eggs and nymphs were present on all three samples, but evidence of *T. radiata* parasitism was found only on one sample, on which estimated parasitism of nymphs (including both intact mummies and mummies with exit holes) was 91.36%. One set of samples from Nogales was also shipped and processed. This shipment contained 14 samples from seven sites, which were collected in early November.

Table 1. Average adult ACP (±SE) per two-minute count (October; marked with an asterisk) or tap sample (November and December).

	October 19*	November 19	December 19
San Luis	0	0	0
Wellton	0	0	0
Ajo	5.36 ± 1.60	1.42 ± 0.38	No count
Nogales	5.52 ± 0.90	1.26 ± 0.26	0.77 ± 0.16

Table 2. Percent of sampled branches with ACP eggs and/or nymphs present.

	October 19	November 19	December 19
San Luis	0	0	0
Wellton	0	0	0
Ajo	5.13%	1.04%	No count
Nogales	7.56%	8.93%	21.88%

Parasitism ranged from zero to 64.20%, with an average of 11.29% parasitism.

The *T. radiata* collected during pre-release monitoring in Nogales were determined to all belong to a single COI haplotype, one which is not found among the Pakistani-origin isolines reared at UCR. This haplotype is, however, represented in *T. radiata* being mass-reared and released in Mexico.

Conclusions

Releases of *T. radiata* will continue along with ACP and parasitoid population monitoring. Seasonal fluctuations in citrus flush growth and ACP populations will be tracked to establish patterns. Long-term population trends as well as parasitism rates will also be examined to look for evidence of *T. radiata* establishment and impact from biological control; however, as activities at new sites began only in fall, results of *T. radiata* releases in these areas will not be apparent for some time.

Periodic samples of *T. radiata* will also be taken in each region for genetic analysis to evaluate whether genetic diversity from the source material is being maintained and, in the case of the Nogales sites, to determine what proportion of *T. radiata* present are descended from the Pakistani-origin wasps.

Reference

1) 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;48(3):514-23.

Area-wide control of Asian Citrus Psyllid in urban-commercial citrus buffers in Southern California

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Introduction

Candidatus Liberibacter asiaticus (CLas), the causal agent bacterium of Huanglongbing (HLB) disease of citrus, also known as Citrus Greening, was first detected in California in 2012. It is vectored by the Asian citrus psyllid (ACP), *Diaphorina citri*, which is widely distributed in southern Asia, and was first detected in California in 2008. As one of the principle vectors of CLas, ACP is a key citrus pest. Since the first detection, CLas has been found in more than 1,800 trees in urban backyards at several locations within Los Angeles, Orange, Riverside, and San Bernardino Counties. To date, there have been no detections in commercial orchards. Citrus production in Florida has declined by 50% or more since the pathogen was first detected and the pathogen has devastated millions of acres of citrus crops worldwide. HLB is considered one of the most serious citrus diseases in the world. Once a tree is infected, the fruit production and quality decline until the trees ultimately die.

The HLB-ACP control program in California is a statewide cooperative program led by the California Department of Food and Agriculture (CDFA) and made up of state, county, industry, university, and federal agricultural authorities. The program has prioritized keeping commercial citrus free of HLB until a long-term management solution can be found. The program has focused on three key strategies: 1) the detection of HLB diseased trees, 2) area-wide management to suppress ACP population both in quarantine and generally infested areas in Southern California, and 3) eradication of ACP in areas where it is not well established.

CDFA is leading a statewide ACP control to apply area-wide pesticide treatments to suppress psyllids within a 1/4-mile buffer area of commercial citrus and quarantine zones. There are questions about the long-term effectiveness of these treatments as well as concerns about cost and sustainability. Continued applications of pesticides in these urban areas can be problematic due to rejection by some residents, logistical limitations of long-term applications in residential areas, and limits to maintaining adequate control with the number of applications that are practical over a year. Two years ago, a lawsuit against the CDFA led to the suspension of pest control treatments for ACP and other program pests for over a year, consequently leaving these buffer areas untreated during this time.

CDFA also leads the program to release and establish the ACP specialist parasitoid *Tamarixia radiata* within all ACP infested areas across the state. In the southern region of the state, the strategy focuses on releasing *T. radiata* in HLB quarantine areas, along trade pathways, near the Mexico border. *Tamarixia radiata* has successfully established at 91% of survey sites, including numerous locations distant from release sites. Parasitism levels of ACP by *T. radiata* as high as 60% have been observed, with overall control levels as high as 99% when other natural enemies are present along with *T.radiata* [1,2]. However, monitoring has indicated average ACP parasitism by *T. radiata* is low to moderate (up to 18.6%), with parasitism and natural enemy activity observed to be higher in the absence of interference by the invasive Argentine ant, *Linepithema humile* [2].

Argentine ants and other sugar feeding ants can cause significant disruption of natural control of ACP by *T. radiata* and other natural enemies [1, 3, 4, 5, 6]. For more effective biological control, the University of California IPM recommends that homeowners and growers apply ant controls [4, 5]. Ant control treatment has proven to be effective in residential and small organic citrus plots but has yet to be observed on a more extensive, area-wide basis [7, 5]. Using area-wide tactics (treating several properties rather than a single property) to increase control of Argentine ant in residential areas would be more effective since these species have high populations in urban areas and are highly dispersive.

Project goals

The purpose of this two-year project is to demonstrate and evaluate the use of an Area-Wide Integrated Pest Management (AW-IPM) strategy for the control of ACP by combining multiple biocontrol tools into a single, area-wide control effort. A successful outcome for this project will allow the HLB-ACP program to assess the feasibility of implementing AW-IPM in an existing ACP pest control district's operations, while also determining which tactics, or combination of tactics, can be the most effective. Successful implementation of this demonstration project may help set the basis for a future buffer treatment program in high ACP and high HLB risk areas next to commercial citrus and may allow the replacement of pesticide treatments by other AW-IPM tactics in the event these treatments become unsustainable.

A secondary project goal is to establish commercial production of new species identified as effective ACP predators through work previously funded by the Citrus Research Board (CRB) and Citrus Health Research Program (CHRP). Three predators have been identified by previous research as the most promising to control ACP and have been evaluated in laboratory, greenhouse, and field cage experiments. These include green lacewing, *Chrysoperla Comanche*, and the coccinellid beetles, *Diomus pumilio* and *Rhyzobius lophanthae* [9]. Unpublished data from Gebiola et al. have shown that all three predators are effective biocontrol agents of ACP. To provide material for this project, *C. comanche* and *R. lophanthae* are in commercial production by two producers and *D. pumilio* are being produced by our CRB cooperators.

Project development

An extensive 5-week campaign was conducted in Hemet by a crew of four to six workers to explain the project goals to residents and gain their approval for accessing desired treatment areas. An outreach document was developed with the Hemet Citrus Pest Control District with support from CDFA and the Riverside County Agricultural Commissioner's office (Appendix 1); the document was provided in English and Spanish to ensure broad accessibility.

We collaborated with heayconnect, an agricultural data management company, to develop an application for tablets and smartphones (iOS and Android operating systems) that uses GPS to record monitoring and location data from project sites. The application was developed to aid in the collection and rapid uploading of field data (Figure 1).

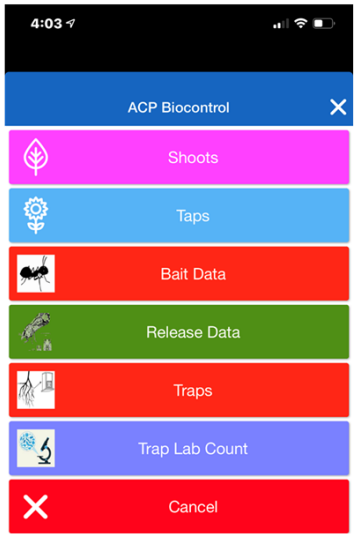

Figure 1. The application developed for collecting data for the area-wide ACP control project. The application allows users to 1) collect and enter data with GIS coordinates, 2) output maps and CSV data files, and 3) manage sampling quality. Additionally, the application saves time by enabling researchers to manage data remotely, and increase the accuracy of field data collection through QR coded trees.

Project design

The demonstration project consists of three treatments: 1) augmentative release of all available natural enemy species, 2) area-wide ant control only, and 3) a control no-treatment area. An additional fourth treatment, the release of natural enemies plus area-wide ant control, may be added in year two if enough properties can be added to the study.

The natural enemy releases include the releases of *D. pumilio, C. comanche,* and *R. lophanthae* at the rate of 20 per tree and the release of *T. radiata* at the rate of 25 per tree, each two times per month. The ant control treatments consists of the application of 1% boric acid in 25% sugar solution [10] in a 1 L reservoir bait station fixed to a tree at the rate of 1 per 10 citrus trees, per residential property (Figure 2). Bait stations are refilled and maintained once per month.

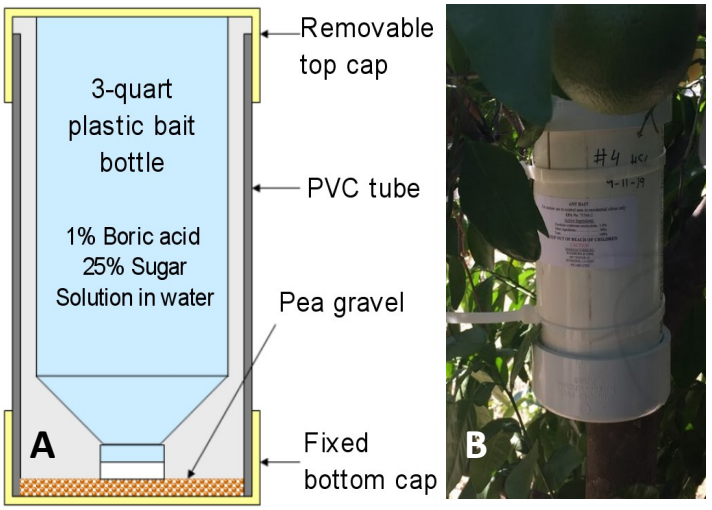

Figure 2. Ant bait stations with 1% boric acid for ant control treatments. A) Schematic showing bait station design. B) Placement of bait station on citrus host tree.

These treatments are deployed in two areas next to a 3,050-acre citrus production area in Hemet, California. The first area is within the ¼ mile buffer next to the commercial citrus groves where CDFA is treating citrus trees twice per year. The second area is a ¼ mile-wide strip adjacent to the outside of the treatment buffer, with the experimental treatments applied to both sides of the treatment line (Figure 3). With this sampling regime, we can contrast differences in treatment effects with and without pesticide applications.

Monthly ACP population monitoring consists of 1) yellow sticky traps deployed at the rate of 1 per property or 1 per 10 trees, 2) tree tap samples counted for adult ACP, predators, and ants at the rate of 1 per 5 trees, and 3) sampling for citrus flush stages and monitoring shoot samples to determine rates of parasitism by *T. radiata* at the rate of 1 per 10 trees.

Figure 3. Map showing the design and layout of the area-wide demonstration project. The red line shows the division between the buffer CDFA pesticide treatment area (inside) and the outside untreated area without pesticide treatment.

Accessing project impact

It is important to note that large-scale demonstration projects are challenging to replicate, and can not be evaluated by-standard statistical methods. However, if robust trends are observed we feel confident that both the large scale (treatment size and participating properties (n = 166) and scope (two-year time period) of the project will allow us to assess the meaningful impacts of the treatments. The findings of this demonstration project can be made more robust by making measurements within the treatment areas to assign impacts. For example, counting the density of ants in treated vs. untreated areas can be related to impacts of ACP density, parasitism rates, or predator abundance. Additionally, to the extent that other similar demonstration projects can be mounted, these can serve over time as a kind of spatial and temporal replication.

Preliminary results

The study area contains a total of 1,107 individual properties. Of these, 560 (51%) have citrus hosts; 166 (30%) residents agreed to participate in the study. These properties have 712 citrus trees representing 46% of the total number of trees (Table 1).

Table 1. Summary of households inside and outside of the residential citrus buffer treatment zone next to commercial citrus that were contacted for the area-wide IPM stud

Properties	N	% of total with Citrus	Pesticide zone	
			In	Out
Total properties	1,107	N/A	535	571
Properties with Citrus	560	100	269	292
Agreed to participate	166	30	92	74
Not participating	394	70	95	97
Citrus hosts				
Total hosts	1555		997	558
# Hosts on participating properties	712	46	514	208

Releases of predators and *T. radiata* in the biological control treatment areas started during the month of September 2019, with twice-monthly releases of most species being made as they were available. Target release numbers for *T. radiata, C. Comanche,* and *R. lophanthae* have been met for most months (Table 2). The release numbers for *D. pumilio* have not been meeting target goals due to a shortage of host plants required for their rearing (Table 2).

Ant bait stations were placed on 59 properties initially at the rate of 1 per 10 trees, for a total of 67 bait stations. Monitoring during the first few months suggested that treatments at this rate were not effective, therefore, the current rate has been doubled to 1 per 5 trees for a total of 134 bait stations.

Monitoring of ACP adults in treatment areas began in August using yellow sticky panel traps. A total of 203 traps were distributed over all treatment areas. Monthly trap counts show that from the beginning, the ACP density was lower for all treatment plots in the inside buffer area compared to the outer area, with populations declining during the fall months for both areas (Figure 4).

Table 2. Numbers of biological control agents released inside (In) and outside (Out) of the residential citrus buffer treatment zone, monthly (as of March 2020).

Spp.	Spray zone	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Total
T. radiata	In	0	12,670	5,825	10,450	14,095	6,349	4,650	**54,039**
	Out	2,650	3,050	3,350	3,175	3,380	4,000	1,600	**21,205**
D. pumilio	In	100	920	0	0	20	0	0	**1,040**
	Out	1,060	0	0	0	0	0	0	**1,060**
R. lophanthae	In	0	0	6,960	10,440	11,850	11,850	2,200	**43,300**
	Out	0	0	2,060	2,540	3,340	3,520	1,280	**12,740**
C. comanche	In	2,320	6,340	4,230	8,040	11,600	8,620	3,720	**44,780**
	Out	1,060	1,220	2,000	2,540	3,340	3,520	1,010	**14,690**

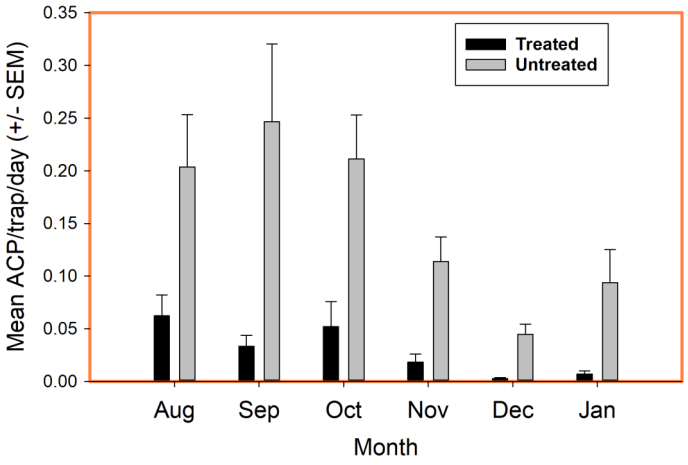

Figure 4. Mean number of ACP per trap per day in outside untreated areas versus inside treated area. Data for treatment areas are pooled into treated and untreated areas. The application of beta-cyfluthrin was applied in September 2019.

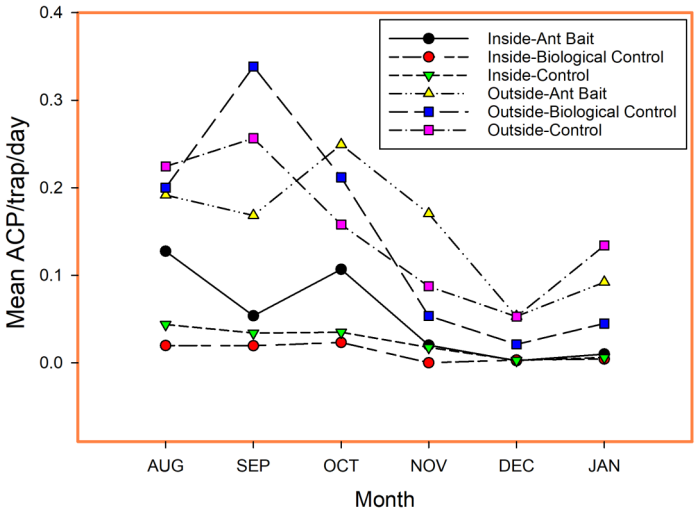

Figure 5. Mean number of ACP per trap per day by treatment in outside untreated areas versus inside treated areas. Beta-cyfluthrin was applied in September 2019.

Trap counts were lower in the inside area by about an order of magnitude. During the second week of September, a beta-cyfluthrin application was made to approximately 90% of the citrus hosts in the inside area.

So far, the AW-IPM plot treatments have not resulted in any discernable impact on adult ACP density, though there was a trend of a decline in ACP numbers on traps during the fall and winter months in the outside biological control treatment area (Figure 5). Similar to the trap data, adult ACP densities from tree tap sampling also show a large difference between the inside and outside areas, as well as a decline in both areas during fall and winter months (Figure 6). There is also a trend for ACP population density decline in the outside biological control treatment (Figure 6).

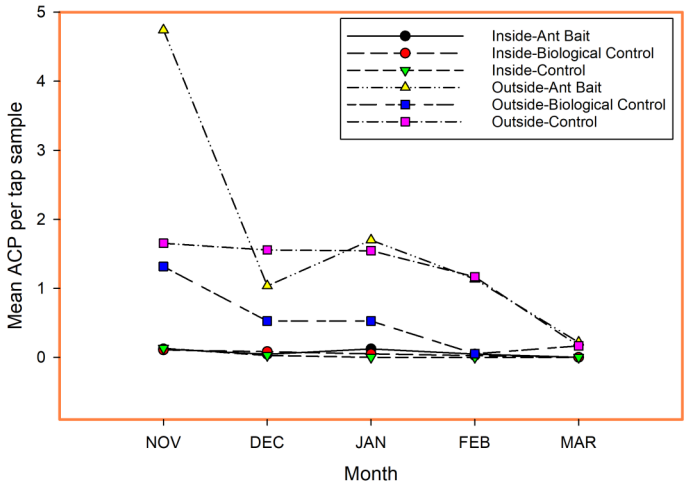

Figure 6. Mean number of ACP per tree tap sample by treatment in outside untreated areas versus inside treated areas. Beta-cyfluthrin was applied in September 2019.

Conclusion

This project has been in full operation for approximately four months. All biocontrol treatment areas are receiving regular releases, as planned, and all the ant bait stations have been deployed. Thus far, there has not been a substantial difference in ACP density between treatments. There likely has not been enough time for these treatments to start to show any effects, especially as the cooler temperatures reduce ACP reproduction rates, and few ACP and other insect hosts are available for predator and parasitoid populations to build up.

It is unclear whether the ant treatments are having any impacts as of yet. No differences in ACP densities were shown between treatments for most sampling dates nor ant counts (data not shown). We chose a boric acid ant bait placement rate of 1 per 12 trees, which was derived from the literature to be roughly equivalent to 1 per 12 trees used in commercial citrus groves. This rate may not be directly transferable to a residential setting where there are far lower densities of citrus trees compared to citrus groves because other sources of both food and non-ACP hemipterans tended by Argentine ants may be present. Because there may be properties nearby without citrus that also have colonies of Argentine ants, our treatment rate may not be effective in limiting the influx of ant migration from outside of the treatment area. For these reasons, we doubled the treatment rate to one bait station per five trees and are also looking at other methods to apply more effective area-wide treatment and to evaluate their impacts.

The biggest finding so far has been the differences in ACP populations between the inside buffer area treated with pesticides and the outside untreated areas. We observed these

differences in ACP densities between the two areas before the September pesticide applications, and these differences have persisted, ranging from 10 to 20 times lower in the inside buffer area next to the commercial groves. To our knowledge, these are the first data collected regarding the efficacy of the statewide program. Based on results from research conducted in Florida, these levels are low enough to limit the establishment of HLB disease and suggest that the buffer treatment program can be highly effective.

It is unknown why the inside area had much lower ACP populations compared to the outside of the treatment line in residential citrus just ¼ mile away before pesticide applications were resumed in September. The State program was suspended in the spring of 2018, and this area had not received treatment by the State program for more than a year. During this lapse of treatments, we did learn that the Hemet pest control district was able to treat some of the residential properties with larger amounts of citrus, which likely had some impact. Also, the commercial citrus area adjacent to the inside buffer area was receiving treatment of up to three times per year, which would limit ACP migration into the zone. Finally, the residual effect of previous treatments from systemic treatment may be having an impact for longer than expected. This study will continue for another year and a half. Our goal remains to determine if the AW-IPM approach described in this report can be integrated with area-wide residential treatments. Findings from this two-year demonstration project will be used to make program recommendations to California's cooperative HLB-ACP control program on potential new ACP control methods. Other projects with additional cooperators are being planned to obtain more data from other sites with large-scale pesticide treatments and releases of *T. radiata* to add to the HLB/ACP program's capacity to evaluate the impacts of these programs.

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Appendix

Help Save Hemet's Citrus Trees

In cooperation with the University of California and the United States Department of Agriculture, the Hemet Citrus Pest Control District is developing a tool box of options to control a dangerous insect called the Asian citrus psyllid (ACP) – and we need your help.

ACP spreads a devastating citrus disease called Huanglongbing, or HLB. HLB kills all citrus trees it infects, and while it hasn't been found in Hemet yet, the disease is spreading in Los Angeles, Orange and western parts of Riverside counties. The Hemet Citrus Pest Control District and local citrus growers are making an area-wide effort to control the ACP and evaluating various methods of pest control in residential citrus trees.

You can help by:

- Allowing crews from the California Department of Food and Agriculture to inspect and treat your citrus trees. This is the most effective method for lowering ACP populations.

- Notifying the pest control district of your interest in having beneficial insects released on your property. Beneficial insects provide a natural way to control citrus pests – like ACP – as well as other garden pests, through a tactic known as biological control. These small, beneficial insects only feed on other insects and are not a nuisance to people or pets.
  - If interested, our project crew will need to have access to your property during weekdays to monitor for pests on your citrus trees, release beneficial insects, set up an ant control station and hang one yellow panel trap used to catch insects on your citrus trees. On average, one or two project people will be on your property once a week throughout the year.
  - Email hemetareawideacpproject@gmail.com if you are interested in having beneficial insects and control for ants on your property.

BESIDES HELPING TO PROTECT HEMET'S CITRUS TREES, THE BENEFITS WILL INCLUDE:

1) Control of ants on your property. Some areas will be provided with a white plastic pipe ant control station which will help control Argentine ants (or kitchen ants) that are often a nuisance in gardens and homes. These ants actually protect several pests, like ACP, from beneficial insects. One station will be secured to one citrus tree at about 5 feet above the ground out of reach of kids or pets.

2) A chance to observe science at work in your own garden and to learn about the natural history of pests and the beneficial species that eat them.

For more information:
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Appendix 1. A resident outreach document from Hemet Pest Control District for Area-Wide ACP control project.

Otis Laboratory members are indicated in bold

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